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Original article

Multivariate morphometric analysis of *Apis cerana* of southern mainland Asia

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Abstract – Multivariate morphometric analyses were performed on a series of worker honeybees, *Apis cerana*, representing 557 colonies from all of southern mainland Asia extending from Afghanistan to Vietnam south of the Himalayas. Scores from the principal components analysis revealed five statistically separable but not entirely distinct morphoclusters of bees: (1) the Hindu Kush, Kashmir, N. Myanmar, N. Vietnam and S. China; (2) Himachal Pradesh region of N. India; (3) N. India, Nepal; (4) central and S. Myanmar and Vietnam, Cambodia, Thailand, S. China and peninsular Malaysia; (5) central and S. India. The major morphoclusters are distributed coherently with the different climatic zones of the region. While populations are definable, nomenclatural adjustments remain for the future.

***Apis cerana* / honeybees / subspecific taxon / morphocluster / morphometry / southern mainland Asia**

1. INTRODUCTION

A recent analysis of the infraspecific categories of *Apis cerana* Fabr. established that their classification is in a state of flux and uncertainty (Hepburn et al., 2001a). To resolve these problems, systematic and multivariate analyses of all available and newly gathered morphometric data for *A. cerana* are being undertaken. This will allow the species to be evaluated as a contiguous population using the same criteria and methods of approach across its full range of distribution. Thus far, in western Asia *A. cerana* populations extending from the Hindu Kush of northern Afghanistan and Pakistan (Radloff et al., 2005) and along the southern Himalayas through India and Nepal (Hepburn et al., 2001b) have been analyzed and their morphocluster distributions and boundaries determined.

In eastern Asia, *A. cerana* populations of both Thailand and Malaysia have recently been analyzed morphometrically (Limbipichai, 1990; Damus, 1995; Damus and Otis, 1997; Sylvester et al., 1998). However, the absence of morphometric data from *A. cerana* of Myanmar has precluded an analysis of the *A. cerana* of the western sector of Southeast Asia. Likewise, excepting limited data from Vietnam (Ruttner, 1988), Indochina remains unexplored. Recent collections of *A. cerana* in Myanmar, Thailand, Cambodia and Vietnam

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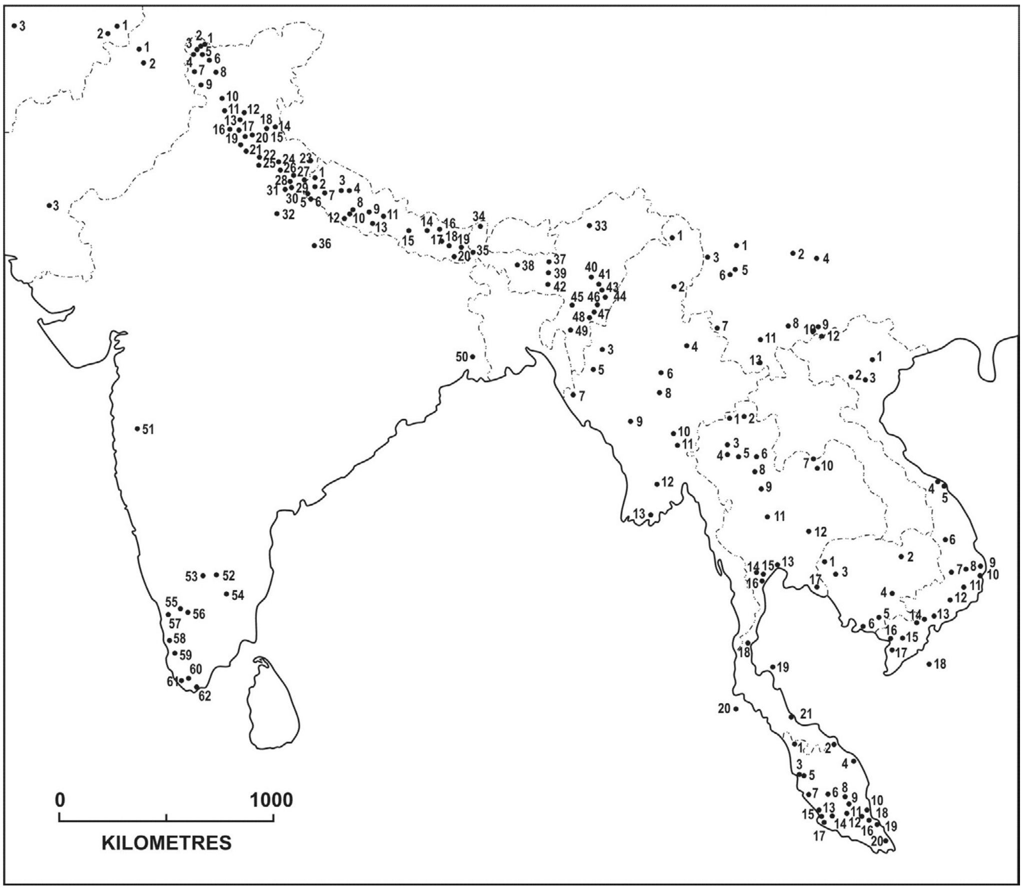

Figure 1. Geographical distribution of localities for *Apis cerana* in southern mainland Asia (additional details in Tab. I).

have greatly augmented a growing database for this species in Southeast Asia. Here we report the results of multivariate morphometric analyses of *A. cerana* for the entire southern mainland of Asia south of the Himalayas extending from the western border of *A. cerana* distribution in Afghanistan to its southeastern limits in Vietnam.

2. MATERIALS AND METHODS

2.1. Honeybees

The worker honeybee specimens used for analysis derive from several different sources. (1) New material has been collected throughout Myanmar, Malaysia, Cambodia and Vietnam. (2) Raw data from Thailand, Vietnam, Malaysia, and Myanmar in the *A. cerana* database of the Institut für Bienenkunde at Oberursel (most previously analyzed by Ruttner, 1988) are included here. (3) The raw databases of previously published results for India and Nepal (Singh and Verma, 1993; Singh et al., 1990; Verma et al., 1989; 1994; Hepburn et al., 2001b) and for Afghanistan and Pakistan (Radloff et al., 2005) were also incorporated to provide a single database for a complete analysis of the contiguous populations of *A. cerana* in sub-Himalayan mainland Asia. (4) Similarly, data from recent work on the *A. cerana* of Yunnan Province (Ken et al., 2003) are included here. The geographical origins of 8424 worker honeybees of *A. cerana* from 557 colonies at 179 localities used in the current analysis are listed in Table I and shown in Figure 1.

Table I. Geographical origins, altitude and colony sample sizes (n) of *A. cerana* used in the morphometric analysis of honeybees of southern mainland Asia.

Locality and Map ref. Nos.	Coordinates	Alt.	n	Sources	G 1	G 2	G 3
AFGHANISTAN							
1. Bidiwa	34.56N 70.22E	2206	4	Oberursel; Radloff et al., 2005	?	1	1
2. Kabul	34.31N 69.08E	1795	2	Oberursel; Radloff et al., 2005	1	1	1
3. Anhangaran	34.30N 64.51E	2062	2	Oberursel; Radloff et al., 2005	1	1	1
PAKISTAN							
1. Peshawar	34.00N 71.32E	317	7	Oberursel; Radloff et al., 2005	1	1	1
2. Bulchok/Swat	33.33N 71.53E	349	1	Oberursel; Radloff et al., 2005	?	4	?
3. Thari	27.13N 68.33E	41	1	Oberursel	4	4	?
INDIA							
1. Gurais	34.37N 74.53E	2364	5	Hepburn et al., 2001b	1	1	1
2. Kupwara	34.31N 74.16E	1811	5	Hepburn et al., 2001b	1	1	1
3. Dras	34.26N 75.46E	2977	4	Hepburn et al., 2001b	1	1	1
4. Sonamarg	34.18N 75.21E	2740	6	Oberursel; Hepburn et al., 2001b	1	1	1
5. Srinagar	34.08N 74.50E	1768	5	Hepburn et al., 2001b	1	1	1
6. Tral	33.56N 75.10E	2007	5	Hepburn et al., 2001b	1	1	1
7. Rajouri	33.23N 74.21E	938	5	Hepburn et al., 2001b	1	1	1
8. Kishtwar	33.19N 75.48E	1664	6	Oberursel; Hepburn et al., 2001b	1	1	1
9. Katra	33.03N 74.57E	753	1	Oberursel	?	1	1
10. Dalhousie	32.32N 75.58E	2036	5	Hepburn et al., 2001b	2	2	2
11. Kangra	32.05N 76.16E	700	5	Hepburn et al., 2001b	2	2	2
12. Katrain	32.05N 77.08E	1463	5	Hepburn et al., 2001b	2	2	2
13. Mandi	31.43N 76.50E	761	5	Hepburn et al., 2001b	2	2	2
14. Pooh	31.40N 78.34E	2837	5	Oberursel; Hepburn et al., 2001b	2	2	2
15. Roghi	31.32N 78.15E	3017	5	Hepburn et al., 2001b	2	2	2
16. Bhareri	31.23N 76.34E	1007	5	Hepburn et al., 2001b	2	2	2
17. Bilaspur	31.15N 76.40E	587	5	Hepburn et al., 2001b	2	2	2
18. Bagi	31.15N 77.27E	2648	5	Hepburn et al., 2001b	2	2	2
19. Shimla	31.07N 77.10E	2206	5	Hepburn et al., 2001b	2	2	2
20. Solan	30.50N 77.08E	1530	5	Hepburn et al., 2001b	2	2	2
21. Nahan	30.33N 77.21E	905	5	Hepburn et al., 2001b	2	2	2
22. Dehradun	30.30N 78.08E	762	4	Hepburn et al., 2001b	3	3	3
23. Gubudia	30.21N 80.32E		1	Oberursel	4	4	?
24. Pauri	30.12N 78.48E	1550	2	Hepburn et al., 2001b	2	2	?
25. Haridwar	30.02N 78.08E	330	2	Hepburn et al., 2001b	3	3	3
26. Chaubattia	29.55N 79.00E	212	4	Hepburn et al., 2001b	?	3	3
27. Almora	29.36N 79.40E	1651	5	Hepburn et al., 2001b	3	4	?
28. Lohaghat	29.25N 80.06E	1754	5	Hepburn et al., 2001b	3	3	3
29. Nainital	29.23N 79.32E	1920	2	Hepburn et al., 2001b	?	2	3
30. Jeolikote	29.16N 79.46E	1250	5	Hepburn et al., 2001b	?	3	3

Table I. Continued.

Locality and Map ref. Nos.	Coordinates	Alt.	n	Sources	G 1	G 2	G 3
31. Haldwani	29.13N 79.31E	424	5	Hepburn et al., 2001b	3	3	3
32. Budaun	28.02N 79.07E	169	5	Hepburn et al., 2001b	3	3	3
33. Itanagar	27.08N 93.40E	440	2	Hepburn et al., 2001b	3	3	3
34. Gangtok	27.02N 88.40E	1818	3	Hepburn et al., 2001b	3	3	3
35. Kurseong	26.56N 88.20E	1458	3	Hepburn et al., 2001b	3	3	3
36. Lucknow	26.50N 80.54E	131	5	Hepburn et al., 2001b	?	3	5
37. Khoirabari	26.38N 91.51E	134	5	Hepburn et al., 2001b	3	3	3
38. Bongaigaon	26.28N 90.32E	76	3	Hepburn et al., 2001b	?	3	3
39. Guwahati	26.11N 91.47E	56	2	Hepburn et al., 2001b	?	3	3
40. Dimapur	25.54N 93.48E	134	2	Hepburn et al., 2001b	?	3	3
41. Kohima	25.41N 94.06E	1261	6	Hepburn et al., 2001b	3	3	3
42. Shillong	25.34N 91.56E	1496	3	Hepburn et al., 2001b	3	3	3
43. Mao	25.30N 94.07E	1470	3	Hepburn et al., 2001b	3	3	3
44. Ukhrul	25.08N 94.23E	1662	5	Hepburn et al., 2001b	3	3	3
45. Silchar	24.50N 92.51E	22	3	Hepburn et al., 2001b	?	3	3
46. Imphal	24.49N 93.58E	786	5	Hepburn et al., 2001b	3	3	3
47. Moirang	24.30N 93.48E	766	5	Hepburn et al., 2001b	?	3	3
48. Churachandpur	24.20N 93.40E	922	4	Hepburn et al., 2001b	3	3	3
49. Aizawl	23.45N 92.45E	1018	5	Hepburn et al., 2001b	3	3	3
50. Calcutta	22.34N 88.21E	13	1	Oberursel	?	4	?
51. Poona	18.32N 73.48E	570	3	Oberursel	?	4	?
52. Bangarapet	12.58N 78.12E	61	5	Verma (unpublished)	5	5	5
53. Bangalore	12.58N 77.34E	1458	5	Verma (unpublished)	5	5	5
54. Yarakapadi	12.18N 78.38E		5	Oberursel	?	4	?
55. Ooty	11.24N 76.42E	2214	5	Verma (unpublished)	?	5	5
56. Coonoor	11.21N 76.49E	1502	5	Verma (unpublished)	5	5	5
57. Nilambor	11.16N 76.15E	61	5	Verma (unpublished)	5	5	5
58. Ernakulam	9.58N 76.16E	3	5	Verma (unpublished)	5	5	5
59. Kottayam	9.35N 76.31E	3	5	Verma (unpublished)	5	5	5
60. Pon Mudi	8.44N 77.04E	134	5	Verma (unpublished)	5	5	5
61. Trivandrum	8.28N 76.55E	0	2	Verma (unpublished)	?	5	5
62. Kanyakumari	8.05N 77.34E	0	5	Verma (unpublished)	5	5	5
NEPAL							
1. Lali	29.49N 80.36E	1097	3	Hepburn et al., 2001b	?	2	3
2. Lanakedareshwar	29.22N 80.56E	1360	3	Hepburn et al., 2001b	?	3	3
3. Ghughuti	29.18N 82.12E	2470	5	Hepburn et al., 2001b	?	2	3
4. Vinaula	29.18N 82.18E	2895	5	Hepburn et al., 2001b	1	1	1
5. Navadurga	29.15N 80.27E	1837	5	Hepburn et al., 2001b	?	2	3
6. Sharmali	29.14N 80.25E	1635	3	Hepburn et al., 2001b	?	2	3
7. Durg	29.10N 81.20E	1345	5	Hepburn et al., 2001b	3	3	3
8. Liwang	28.25N 82.40E	1500	5	Hepburn et al., 2001b	?	3	3

Table I. Continued.

Locality and Map ref. Nos.	Coordinates	Alt.	n	Sources	G 1	G 2	G 3
9. Jumla	28.24N 83.33E	2662	2	Oberursel	?	1	1
10. Laltibang	28.20N 82.30E	900	5	Hepburn et al., 2001b	?	3	3
11. Lumle	28.20N 84.00E	1400	5	Hepburn et al., 2001b	?	3	3
12. Ghorahi	28.05N 82.20E	400	5	Hepburn et al., 2001b	6	4	6
13. Tansen	27.55N 83.40E	1067	5	Hepburn et al., 2001b	3	3	3
14. Suspa	27.44N 86.10E	1600	5	Hepburn et al., 2001b	3	3	3
15. Kathmandu	27.41N 85.16E	1344	3	Oberursel	?	2	?
16. Nawakot	27.41N 85.43E	743	1	Oberursel	4	4	?
17. Sidheshwar	27.18N 85.56E	1463	3	Hepburn et al., 2001b	3	3	3
18. Homtang	27.10N 87.10E	600	5	Hepburn et al., 2001b	7	4	7
19. Virgaon	27.05N 87.51E	1200	5	Hepburn et al., 2001b	?	4	?
20. Letang	26.40N 87.25E	250	5	Hepburn et al., 2001b	6	4	6
MYANMAR							
1. Putao	27.22N 97.27E	534	5	This study	?	1	1
2. Myitkyina	25.24N 97.25E	158	5	This study	4	4	4
3. Kalay	23.14N 94.00E	85	5	This study	4	4	4
4. Lashio	22.58N 97.48E	837	5	This study	4	4	4
5. Hakha	22.38N 93.39E	1867	5	This study	4	4	4
6. Pyin-oo-Lwin	22.02N 96.35E	1028	5	This study	4	4	4
7. Paletwa	21.25N 92.49E	88	5	This study	?	4	4
8. Ywa-ngan	21.10N 96.25E		5	This study	?	4	4
9. Magwe	20.08N 94.55E	50	5	This study	4	4	4
10. Thaton	20.06N 96.13E	25	5	This study	4	4	4
11. Thantaung	19.01N 96.34E	120	5	This study	4	4	4
12. Taikkyi	17.18N 95.57E	5	2	Oberursel	4	4	4
13. Bogale	16.15N 95.23E	7	5	This study	?	4	4
CHINA							
1. Lijian	26.52N 100.13E	105	2	Oberursel	?	1	1
2. Sayingpan	26.01N 102.31E	2279	2	Oberursel	?	1	1
3. Lishui	25.57N 98.49E	63	2	Oberursel	?	1	1
4. Huize	25.55N 103.26E	2218	1	Oberursel	?	1	1
5. Binchuan	25.51N 100.33E	1442	2	Oberursel	1	1	1
6. Yuangbi	25.39N 99.56E	2	2	Oberursel	?	1	1
7. Zhenkang	24.00N 98.55E	868	2	Oberursel	?	2	?
8. Kaiyuan	23.41N 102.05E	1148	2	Oberursel	?	1	1
9. Caoba	23.32N 103.23E	1293	2	Oberursel	1	1	1
10. Pingbian	22.50N 103.43E	1400	2	Oberursel	?	1	1
11. Simao	22.43N 100.56E	1549	2	Oberursel	4	4	4
12. Hekou	22.30N 103.57E	122	2	Oberursel	?	2	1
13. Jianghong	21.55N 100.52E	26	2	Oberursel	?	4	4

Table I. Continued.

Locality and Map ref. Nos.	Coordinates	Alt.	n	Sources	G 1	G 2	G 3
THAILAND							
1. Fang	20.05N 99.30E	471	1	Oberursel	?	4	4
2. Chiang Rai	19.56N 99.51E	391	-	Sylvester et al., 1998			
3. Chiang Mai	18.48N 98.59E	295	-	Sylvester et al., 1998			
4. Lamphun	18.36N 99.02E	284	-	Sylvester et al., 1998			
5. Lampang	18.16N 99.30E	247	-	Sylvester et al., 1998			
6. Phrae	18.07N 100.09E	163	-	Sylvester et al., 1998			
7. Nong Khai	17.52N 102.44E	165	-	Sylvester et al., 1998			
8. Uttaradit	17.38N 100.05E	75	-	Sylvester et al., 1998			
9. Phitsanulok	16.47N 100.16E	44	1	Oberursel; Sylvester et al., 1998	?	4	4
10. Khon Kaen	16.26N 102.49E	182	3	Oberursel; Sylvester et al., 1998	4	4	4
11. Uthai Thani	15.22N 100.03E	22	-	Sylvester et al., 1998			
12. Nakhon Ratchasima	15.00N 102.06E	177	-	Sylvester et al., 1998			
13. Bangkok	13.39N 100.31E	0	2	Oberursel	?	4	4
14. Rat Buri	13.30N 99.50E	6	-	Sylvester et al., 1998			
15. Samut Songkram	13.25N 100.01E	5	-	Sylvester et al., 1998			
16. Phet Buri	13.05N 99.58E	10	-	Sylvester et al., 1998			
17. Chantaburi	12.36N 102.07E	6	1	Oberursel; Sylvester et al., 1998	?	3	?
18. Chumphon	10.30N 99.11E	11	-	Sylvester et al., 1998			
19. Suratthani (Samui Is.)	9.09N 99.20E	2	-	Sylvester et al., 1998			
20. Phuket Is.	7.52N 98.22E	9	-	Sylvester et al., 1998			
21. Songkhla	7.12N 100.35E	15	-	Sylvester et al., 1998			
CAMBODIA							
1. Sisophon	13.37N 102.58E	16	2	This study	4	4	4
2. Stoeng Treng	13.26N 106.00E	99	5	This study	?	4	4
3. Battambang	13.06N 103.13E	40	4	This study	4	4	4
4. Kompong Cham	11.59N 105.26E	21	3	This study	?	4	4
5. Takeo	11.00N 104.46E	8	1	This study	4	4	4
6. Kampot	10.37N 104.11E	21	2	This study	?	4	4
VIETNAM							
1. Hoa Binh	20.49N 105.19E	295	4	Oberursel	2	1	1
2. Moc Chau	20.15N 104.37E	1050	5	Oberursel	1	1	1
3. Cuc Phuong	20.15N 105.58E	656	5	Oberursel	?	1	1
4. Duc Pho	14.45N 109.59E	10	2	This study	4	4	4
5. Tam Quan	14.34N 109.03E	6	3	This study	4	4	4
6. Pleiku	13.57N 108.01E	756	5	This study	4	4	4
7. Buon Ma Thuot	12.41N 108.02E	481	2	This study	?	4	4
8. M'drak	12.37N 108.46E	500	3	This study	4	4	4
9. Ninh Hoa	12.28N 109.07E	12	-	This study			
10. Nha Tranh	12.15N 109.10E	4	5	This study	4	4	4

Table I. Continued

Locality and Map ref. Nos.	Coordinates	Alt.	n	Sources	G 1	G 2	G 3
11. Dalat	11.56N 108.10E	1500	4	This study	4	4	4
12. Bao Loc	11.33N 107.48E	800	5	This study	4	4	4
13. Ho Chi Minh City	10.46N 106.43E	3	5	This study	4	4	4
14. Long Khanh	10.38N 106.21E	53	1	Oberursel	4	4	4
15. Ben Tre	10.16N 106.02E	255	2	This study	4	4	4
16. Can Tho	10.03N 105.46E	2	4	Oberursel; This study	4	4	4
17. Chan Thanh	10.02N 105.47E	2	1	Oberursel	?	3	?
18. Con Dao (S'On Island)	8.45N 106.35E	1500	5	This study	4	4	4
MALAYSIA (mainland)							
1. Kuala Nerang	6.14N 100.53E	55	1	This study			
2. Kota Baharu	6.07N 102.15E	1	1	This study			
3. Serdang Selangor	5.16N 100.34E	157	2	This study			
4. Marang	5.13N 103.14E	1	1	This study			
5. Selama	5.12N 100.40E	53	1	This study			
6. Ringlet (Fraser's Hill)	4.24N 101.22E	1135	2	Oberursel; This study	4	4	4
7. Sitiawan	4.11N 100.42E	1	1	This study			
8. Kampung Tembeleng	4.06N 102.20E	206	1	This study			
9. Jerantut	3.56N 102.16E	109	1	This study			
10. Pekan	3.29N 103.23E	0	1	This study			
11. Temerloh	3.28N 102.25E	34	1	This study			
12. Kemaman	3.28N 103.20E	0	1	This study			
13. Tanjong Karang	3.26N 101.10E	4	1	This study			
14. Kuala Lumpur	3.08N 101.42E	61	1	Oberursel	?	4	4
15. Kajang	2.59N 101.46E	98	1	This study			
16. Pontian	2.45N 103.31E	0	1	This study			
17. Kuala Selangor	2.34N 101.44E	3	1	Oberursel	?	4	4
18. Port Dickson	2.31N 101.48E	0	1	This study			
19. Mersing	2.25N 103.50E	8	1	This study			
20. Kota Tinggi	1.45N 103.53E	6	1	This study			

G1, G2 and G3 indicate the morphocluster clusters into which the colonies from the localities were classified according to the kernel density plots, cluster analysis and discriminant analysis procedures, respectively. Indicates the localities where the colonies are unclassified.

Additional information was obtained from morphometric analyses of *A. cerana* from Thailand and Malaysia (Limbipichai, 1990; Damus, 1995; Damus and Otis, 1997; Sylvester et al., 1998). Only processed data are given in their publications and all of the original raw data have been lost (Otis and Sylvester, personal communications) thus precluding their direct incorporation in the current analysis. However, a modicum of original raw data for several of the same localities and countries cited in these reports are available in the *A. cerana* database compiled by Ruttner at Oberursel. This fortunate coincidence allows a "goodness of fit" comparison to be made for the conclusions reached in Damus and Otis (1997) and Sylvester et al. (1998).

2.2. Measurements

Twelve morphological characters common to the Ruttner/Oberursel and Verma databases and those in the present study (excluding new data from Malaysia) were utilized. Their Ruttner (1988) and Verma et al. (1989, 1994) numbers are given in round and square brackets, respectively, as follows: length of femur (5) [30], length of tibia (6) [31], metatarsus length (7) [32], tergite 3, longitudinal (9) [42], tergite 4, longitudinal (10) [45], sternite 3, longitudinal (11) [49], wax plate of sternite 3, longitudinal (12) [48], sternite 6, longitudinal (15) [50], forewing, longitudinal (17) [5], wing angle B4 (22) [10], wing angle D7 (23) [11] and wing angle G18 (25) [13].

Measurements in the Oberursel, Verma and Hepburn/Radloff databases were made by different individuals and inevitably involve subjective errors of measurement. Therefore, all data were adjusted arithmetically against values in the Oberursel database. This was done by taking the values of different measurers for bees of the same localities as in the Oberursel database (Verma: Sonamarg and Kishtwar in Kashmir, India; Hepburn: Taikkyi/Bogale, Can Tho and Ringlet (Fraser's Hill) in Myanmar, Vietnam and Malaysia, respectively) and proportionally weighting the values so that the colony mean values of the morphometric characters were the same for all of the localities in common. Only minor adjustments were made: size-related character values from the Verma database were all slightly larger (weights ranged from 0.956 to 0.976); angles of venation, on the other hand, were slightly smaller (weights ranged from 1.009 to 1.034); size-related character values from the Hepburn/Radloff database were all slightly smaller (weights ranged from 1.062 to 1.142); no adjustments were necessary for the angles of venation.

2.3. Data analysis

Multivariate statistical analysis of the data included principal components, kernel density, k-means cluster and linear discriminant analyses, analysis of variance, Wilks' lambda statistic for testing significant differences between the multiple means of the characters entered into the discriminant functions and Levene's F statistic procedure for testing heterogeneity of variances (Johnson and Wichern, 1998; Rao, 1998).

3. RESULTS

In a principal components analysis of twelve morphometric characters of worker honeybees from 537 colonies three principal components with eigenvalues greater than one were isolated. PC 1: size-related characters (5–7), (9–12), (15) and (17) with component loadings between 0.64 and 0.96 accounted for 60.8% of the variation; PC 2: angle of venation (25) with component loading 0.81 accounted for 11.5% of the variation; PC 3: angles of venation (22) and (23) with component loadings both 0.72 accounted for 8.4% of the variation. The three principal components accounted for a total of 81.2% of the variation in the data.

Looking at the principal components and kernel density plots using the first and second principal components scores, five morphoclusters (1 to 5) are present (using tension = 0.25, four prominent clusters (1–4) and one less prominent cluster (5)). Two other minor groups (6, 7) are also present and are shown using tension = 0.1 in Figure 2. The colonies within the solid lines for each of the prominent clusters were classified 1 to 7 (Tab. I, column G1). A k-means cluster analysis using five clusters was used to group the unclassified colonies into one of the five main morphoclusters (Tab. I, column G2). A k-means cluster analysis using the F-value as a stopping rule criterion gave the maximum F-value with groupings of 8 clusters. Discriminant analysis procedures were used to obtain the final groupings of the colonies by maximizing the percentages of correct classification of the colonies in the seven morphoclusters (Tab. I, column G3). Those colonies from localities in Afghanistan (Nos. 1–3), Pakistan (No. 1), Kashmir, India (Nos. 1–9), Nepal (Nos. 4, 9), southern China (Nos. 1–6, 8–10, 12), northern Myanmar (No. 1) and Vietnam (Nos. 1–3) formed one cluster (1); those colonies from localities in the Himachal Pradesh region of northern India (Nos. 10–21) formed cluster (2); those colonies from northwestern and northeastern India (22, 25, 26, 28–35, 37–49), Nepal (Nos. 1–3, 5–8, 10, 11, 13, 14, 17) a third cluster (3); central and southern Myanmar (Nos. 2–13), and Thailand (Nos. 1, 9, 10, 13), mainland Malaysia (Nos. 4, 14, 17), Cambodia (Nos. 1–6), Vietnam (Nos. 4–16, 18) and southern China (Nos. 11, 13) formed a fourth cluster (4); those colonies from localities in southern India (Nos. 36, 52, 53, 55–62) formed a fifth cluster (5); clusters (6) and (7) comprised colonies from Nepal (Nos. 12, 20) and (No. 18), respectively (Fig. 2, Tab. I). The graph of the principal components scores shows that the bees of morphoclusters 3 and 4 are smaller in size than those of morphoclusters 1 and 2 but larger than those of morphocluster 5 (Fig. 2, Tab. II). These results confirm the

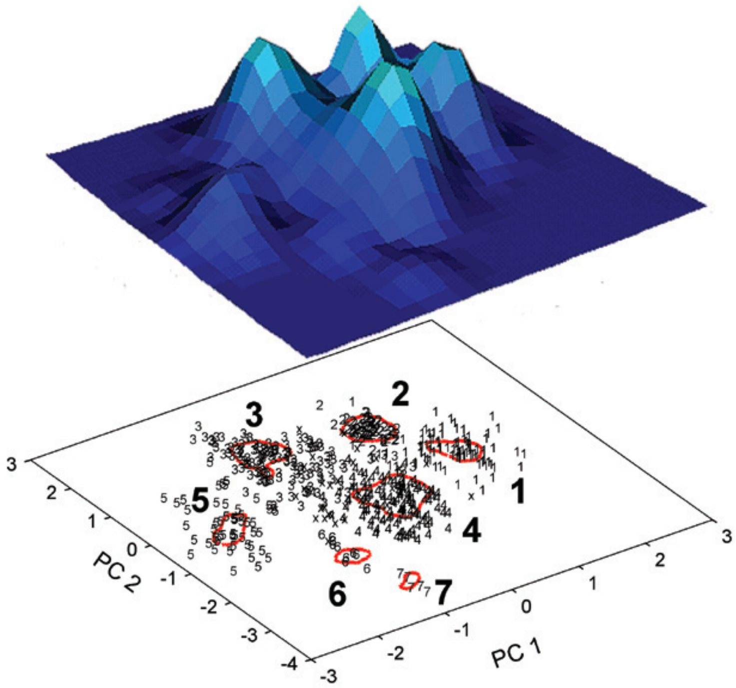

Figure 2. Principal components and kernel density plot of the morphoclusters of *A. cerana* in the southern mainland Asia using 12 morphometric characters: morphocluster 1 comprises colonies from the Hindu Kush, Kashmir, N. Myanmar, N. Vietnam and S. China; morphocluster 2 comprises colonies from Himachal Pradesh, N. India; morphocluster 3 colonies from N.W. and N.E. India and Nepal; morphocluster 4 colonies from central and southern Myanmar and Vietnam, Thailand, Cambodia, S. China and peninsular Malaysia; morphocluster 5 colonies from central and S. India; morphocluster 6 and 7 comprises colonies from Nepal (Nos. 12, 20) and (No. 18), respectively. Solid lines for clusters 1 to 5 drawn with tension = 0.25; for clusters 6 and 7 drawn with tension = 0.1. Numbers indicate the colonies classified by discriminant analysis, x indicates the colonies not classified.

Table II. Means and standard deviations (sd) of 12 morphometric characters measurements in mm, angles in degrees) and n = number of colonies for seven morphoclusters of honeybees of southern mainland Asia.

	Morphoclusters													
	1 n = 100		2 n = 60		3 n = 143		4 n = 132		5 n = 52		6 n = 10		7 n = 5	
character	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
(5)	2.47	0.08	2.35	0.03	2.21	0.05	2.31	0.05	2.04	0.06	2.11	0.02	2.21	0.03
(6)	3.08	0.09	2.99	0.03	2.80	0.06	2.87	0.11	2.55	0.08	2.70	0.03	2.77	0.03
(7)	1.93	0.05	1.88	0.03	1.74	0.04	1.77	0.05	1.60	0.04	1.66	0.02	1.71	0.03
(9)	1.97	0.06	1.88	0.03	1.64	0.16	1.87	0.05	1.51	0.06	1.62	0.01	1.62	0.02
(10)	1.93	0.08	1.81	0.02	1.63	0.06	1.79	0.06	1.48	0.07	1.58	0.03	1.62	0.01
(11)	2.46	0.07	2.31	0.03	2.17	0.07	2.28	0.06	1.94	0.07	2.08	0.02	2.11	0.01
(12)	1.08	0.06	1.36	0.01	0.92	0.09	0.91	0.03	0.88	0.04	0.97	0.01	0.95	0.02
(15)	2.30	0.06	2.23	0.04	2.06	0.06	2.10	0.05	1.91	0.05	2.12	0.02	2.03	0.03
(17)	8.50	0.22	8.30	0.08	8.20	0.29	7.89	0.21	7.28	0.18	7.70	0.07	7.82	0.05
(22)	109.7	2.4	111.1	1.5	111.1	2.8	108.6	3.2	109.6	1.6	106.0	3.6	107.2	0.7
(23)	96.0	1.5	94.6	1.2	94.1	2.2	96.1	1.5	95.9	1.9	94.8	0.5	94.2	0.4
(25)	90.9	2.2	89.9	0.8	89.9	2.3	93.8	1.8	92.0	1.9	99.2	4.3	108.0	1.0

conclusions reached by Ruttner (1988). Significant correlations were found between the principal components 1 scores and altitude (Afghanistan to north western India: r = 0.59, n = 355, $P < 0.001$; Myanmar to Malaysia: r = 0.66, n = 163, $P < 0.001$; Himachal Pradesh: r = 0.88, n = 60, $P < 0.001$; overall: r = 0.47, n = 518, $P < 0.001$).

Kernel density plots using the second and third principal components scores revealed no prominent clusters of the colonies.

The linear discriminant analysis, using the seven morphoclusters correctly classified 100% of the colonies in clusters 1 (n = 100), 2 (n = 60), 4 (n = 132), 5 (n = 52), 7 (n = 5); 98.6% of the colonies (n = 143) in cluster 3 (2 colonies from Tansen (Nepal, No. 13) misclassified into clusters 5 and 6) and 90% of the colonies (n = 15) into cluster 6 (1 colony from Letang (Nepal, No. 20) misclassified into cluster 7). Multiple vector means of the seven morphoclusters were significantly different (Wilks' lambda $\Lambda = 0.0005$; F = 112.9, (72,2639) df, $P < 0.0001$). The means and standard deviations of the twelve characters for each morphocluster are given in Table II. ANOVA procedures revealed significant mean differences between the seven morphoclusters for all twelve characters ($P < 0.0001$).

The localities with mixed classifications of colonies into clusters using discriminant analysis were Almora (India, No. 27), Zhenkang, Kaiyuan, Simao and Jianghong (China, Nos. 7, 8, 11, 13).

The intercolonial variances at the localities with more than one colony were determined using the first principal component loadings. A significant difference was found between the intercolonial variances over all the localities (Levene's F = 3.22 with (144, 392) df, $P < 0.0001$) and significantly high variations were found at some localities: Dehradun (India, No. 22); Kaiyuan, Caoba, Pingbian, Jianghong (China, Nos. 8, 9, 10, 13); Sisophon, Stoeng Treng, Battambang (Cambodia, Nos. 1–3) and Buon Ma Thout, Ho Chi Minh City (Vietnam, Nos. 7, 13).

4. DISCUSSION

The results of the present multivariate morphometric analysis of *A. cerana* of southern mainland Asia yielded five major morphoclusters. This is in sharp contrast to the putative thirteen "subspecies" cited in the historical but mutually incompatible literature on *A. cerana* (Hepburn et al., 2001a). More recently, three additional studies, one on *A. cerana* of the Hindu-Kush region (Radloff et al., 2005), another for the sub-Himalayan foothills (Hepburn et al., 2001b) and a third from Yunnan Province, China (Ken et al., 2003), provided further refinements to *A. cerana* morphoclusters in the region. Predictably, differences between these studies and the present results are apparent. They arise from the effects of interlocality sampling distance and total transect length on morphocluster formation (Radloff and Hepburn, 1998) and from differences in the character suites used in the various studies.

Figure 3 shows the distribution of the morphoclusters obtained in the present study covering all the sub-Himalayan region of mainland Asia from Afghanistan to Vietnam. Before attempting to relate these groups to physiographic variation, it is useful to synonymise the slight differences between the results of the two most recent studies (Hepburn et al., 2001b; Radloff et al., 2005) in relation to the present geographically larger context. In the present case, morphocluster 1 (Fig. 3) has become enlarged and now comprises both morphocluster I, essentially Kashmir, of Hepburn et al. (2001b) and the bees of the Hindu Kush in Afghanistan (Radloff et al., 2005). Likewise, although widely geographically separated from the above, *A. cerana* from northern Yunnan, northern Myanmar and northern Vietnam also belong to the morphocluster 1 group. This result is consistent with another analysis of *A. cerana* from southern China (Ken et al., 2003), and, indirectly with Damus and Otis (1997). The inclusion of all India and Indochina has also modified the morphocluster picture. Morphocluster 2 defined here (Fig. 3) is the same as "morphocluster II" of Hepburn et al. (2001b), while morphocluster 3 equates to "morphoclusters III and IV". The explanation for this modification is that the greater the transect length, the lesser the statistical prominence of many local variations in a greatly enlarged total sample size (Radloff and Hepburn, 1998).

Other morphoclusters shown in Figure 3 are new ones. These include morphocluster 4 which

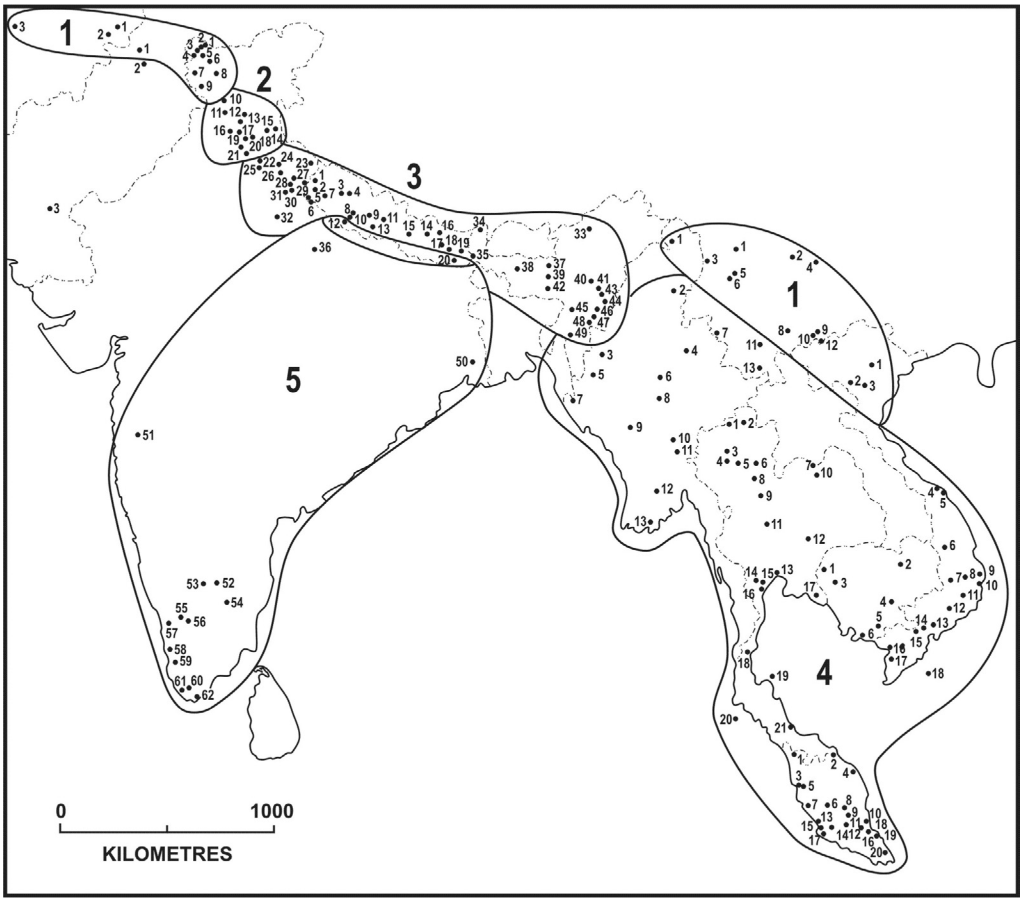

Figure 3. Geographical distribution of principal morphoclusters of *Apis cerana* in southern mainland Asia.

is distributed in Myanmar, southern China, Thailand, Laos, Cambodia, Vietnam and peninsular Malaysia. Morphocluster 5 extends across the central and southern plains of India (Fig. 3). The Damus and Otis (1997) study was principally concerned with oceanic *A. cerana* but they separated the bees of the Malay Peninsula from the former areas.

On the other hand, Sylvester et al. (1998) concentrated specifically on the *A. cerana* of Thailand and peninsular Malaysia and discriminated four morphometric groups, all of which are synonymous with our morphocluster 4. While neither of these studies (Sylvester et al., 1998; Hepburn et al., 2001b) can be faulted on methodological grounds, in both cases they amply demonstrate that these fine discriminations are examples of how character suites, interlocality distance sampling, and more importantly, transect length affect the resolution of continuous populations (Radloff and Hepburn, 1998).

Combining all of the data for a contiguous region into a single analysis, only five major morphoclusters emerged vis-à-vis the disjointed 13 putative subspecies of past years (Hepburn et al., 2001a). The results obtained in the present study are remarkably close to the general conclusions reached by Ruttner (1988) based on a much smaller sample size. These five morphoclusters could be formally designated as "subspecies" but such a course is not recommended, especially in the light of the disjunct geographical distribution for morphocluster 1.

Nomenclatural adjustments should be held in abeyance until all *A. cerana* (including all the *A. cerana* of oceanic Asia and those of China and northeastern Russia) have been analyzed in a composite study, which is now in preparation.

A visual comparison of the morphocluster distributions from this study with climatic zones for southern Asia (Troll and Paffen, 1980; Müller, 1982) demonstrates a general, but by no means quantitative, concordance between them. The general "goodness of fit" between the geographic distributions of the morphoclusters of *Apis cerana* defined here and the major climatic zones of southern mainland Asia suggest a functional relationship between bee morphology and climate/vegetation. The bees of morphocluster 1 are geographically disjunct and each subcluster occupies a climatically different zone: the *A. cerana* of the Hindu Kush and Kashmir subcluster inhabit a region that is cool temperate with a steppe climate, cold winters and arid summers, and vegetation which is dwarf shrub or thorn-steppe; but, those of southwest China occupy a region that is a warm-temperate subtropical zone with dry winters and wooded steppe.

The bees of Himachal Pradesh, India (morphocluster 2) fall within the warm-temperate subtropical zone with a steppe climate, dry winters and steppe thornbush, while in most of Nepal to northeast India (morphocluster 3) the region is tropical. The bees of morphocluster 4 occupy a wet/dry tropical climate to tropical climate and associated tropical forests. The *A. cerana* of morphocluster 5 occur in the great Indian plains region which is tropical but highly variable for aridity.

As we continue our analyses of the other *A. cerana* populations in Asia, it will be interesting to see if this congruence of bee morphotypes and climate/vegetation occurs across all climatic zones. Analyses of these populations of *A. cerana* are currently under review.

Résumé – Analyse morphométrique multivariée d'*Apis cerana* en Asie continentale méridionale. Des analyses morphométriques multivariées ont été réalisées sur une série de 8424 ouvrières d'abeilles *Apis cerana* Fabr., représentant 557 colonies réparties en 179 localités. Les lieux d'échantillonnage étaient distants les uns des autres de moins de 100 km et répartis dans toute l'Asie continentale méridionale située au sud de l'Himalaya, de l'Afghanistan au Vietnam. Les données comprenaient de nouveaux échantillons provenant du Vietnam, du Cambodge, de Malaisie et de Myanmar, ainsi que des données déjà analysées provenant principalement de la base de données d'Oberursel pour *Apis cerana* (Tab. I, Fig. 1) Les mesures ayant été faites par diverses personnes, ces valeurs ont été ajustées par rapport à la base de données d'Oberursel en prenant des paires de mesures pour les mêmes localités et en les ajustant en conséquence.
Les valeurs de l'analyse en composantes principales et des graphiques de densité des noyaux ont montré l'existence de 5 groupes morphologiques statistiquement séparables, mais non totalement distincts : (1) Hindu Kush, Cachemire, Nord du Myanmar, nord du Vietnam et sud de la Chine, (2) région de l'Himachal Pradesh en Inde, (3) nord-ouest de l'Inde, Népal et nord-est de l'Inde, (4) centre et sud du Myanmar, Thaïlande, Cambodge, sud du Vietnam et (5) centre et sud de l'Inde (Fig. 2).
Lorsque l'on compare ces résultats à ceux des autres études morphométriques multivariées récentes portant sur des régions plus petites, des différences apparaissent. Par exemple, *A. cerana* de Thaïlande a été divisée en 4 groupes distincts (Sylvester et al., 1998) et d'autres sous-groupes biométriques ont été signalés dans la région de l'Himalaya (Hepburn et al., 2001b). Cela provient du fait que la formation d'un groupe morphologique est fortement influencée par la taille de la région étudiée, par la liste des caractères utilisés et par la distance entre les points d'échantillonnage. Ainsi la résolution fine des études locales est statistiquement noyée dans une étude à grande échelle. L'analyse de la distribution des groupes morphologiques en termes de zones physiographiques et climatiques montre généralement une bonne convergence entre la morphologie des abeilles et leur environnement caractérisé par le climat et le type de végétation. Il faut attendre que l'analyse d'*A. cerana* dans toute sa zone de répartition soit terminée pour ajuster la nomenclature.

***Apis cerana* / taxon infraspécifique / groupe morphologique / morphométrie / Asie continentale méridionale**

Zusammenfassung – Multivariate morphometrische Analyse von *Apis cerana* des südlichen asiatischen Festlands. Eine multivariate morphometrische Analyse wurde anhand von Messungen an insgesamt 8424 individuellen Arbeiterinnen von *Apis cerana* aus 577 Völkern von 179 Probenorten durchgeführt. Die Studie erfasste mit einer Sammelabstandsauflösung von <100 km den gesamten Bereich des südlichen asiatischen Festlandes einschließlich des südlichen Himalayas von Afghanistan bis Vietnam. Die Daten enthielten neues Material aus Vietnam, Kambodscha, Malaysia und Myanmar sowie zuvor analysierte Daten zumeist aus der Datenbank von Oberursel (Tab. I, Abb. 1). Da die

Messungen von verschiedenen Untersuchern stammten, wurden sie anhand von Messungen der vom gleichen Ort stammenden Bienen gegenüber der Oberurseler Datenbank abgeglichen.

Die Faktorwerte der Hauptkomponentenanalyse und Kerndichteplots zeigten 5 statistisch unterscheidbare, aber nicht gänzlich getrennte Morphokluster von Bienen (1) Hindukusch, Kaschmir, N. Myanmar, N. Vietnam and S. China; (2) die Region Himachal Pradesch von N. Indien; (3) N.W. Indien, Nepal und N.O. Indien; (4) Zentral und S. Myanmar, Thailand, Kambodscha, S. Vietnam, S. China und die Halbinsel von Malaysia; (5) Zentral und S. Indien (Abb. 2). Im Vergleich der gegenwärtigen Resultate mit denen anderer neuerer multivariaten morphometrischen Untersuchungen treten deutliche Unterschiede zutage, insbesondere wenn diese auf kleinere Gebiete bezogen waren. So unterteilte zum Beispiel die Studie von Sylvester et al. (1998) *A. cerana* von Thailand in vier getrennte Kluster, und zusätzliche biometrische Untergruppen wurden in den Regionen des Himalaya ermittelt (Hepburn et al., 2001b). Dies ist darauf zurückzuführen, dass die Ausbildung von Morphoklustern in den Analysen stark von der Größe des Untersuchungsgebietes, der Zusammenstellung der vermessenen Merkmale sowie den Abständen zwischen den Sammelorten beeinflusst wird. Hierdurch geht die hohe Auflösung lokaler Studien in großflächigen Studien verloren. Eine Analyse der Verteilung der Morphokluster in Bezug auf physiographische und klimatische Zonen zeigt generell eine gute Übereinstimmung zwischen der Morphologie der Bienen und ihrer durch das Klima oder die Vegetation gekennzeichneten ökologischen Umgebung. Eine Überarbeitung der Nomenklatur muss zurückgestellt werden, bis *A. cerana* in ihrem gesamten Verbreitungsgebiet erfasst ist.

***Apis cerana* / Honigbienen / Morphokluster / südliches asiatisches Festland**

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