Randall Hepburn
Sarah E. Radloff
*Editors*

# Honeybees of Asia

Springer

Honeybees of Asia

H.R. Hepburn • S.E. Radloff
Editors

# Honeybees of Asia

Springer

*Editors*
Professor Dr. H.R. Hepburn
Department of Zoology and Entomology
Rhodes University
Grahamstown 6140, South Africa
r.hepburn@ru.ac.za

Professor Dr. S.E. Radloff
Department of Statistics
Rhodes University
Grahamstown 6140, South Africa
s.radloff@ru.ac.za

ISBN 978-3-642-16421-7 e-ISBN 978-3-642-16422-4
DOI 10.1007/978-3-642-16422-4
Springer Heidelberg Dordrecht London New York



*Cover illustration:* Pollen forager of *Apis cerana* on an ornamental flower (*Portulaca oleracea*) in the centre of Hangzhou (Zhejiang, China). Photo: Nikolaus Koeniger

*Cover design:* WMXDesign GmbH, Heidelberg, Germany

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

*In Memoriam*

*Eva Widdowson Crane*

*1912–2007*

# Preface

Studies on the biology of honeybees stem from ancient times, in both Asia and Europe. However, published scientific works on the honeybees of both regions gained unanticipated momentum on the heels of World War II and were boosted exponentially by Sputnik a decade later. Since that time, 95% of all publications on Asian and 99% on European honeybees were published. We believe that the publication of the Ruttner's monographs (1988, 1992) was further major stimuli for research on Asian honeybees. Having just brought extraordinary clarity to the "real" honeybees (*Apis cerana*, *Apis dorsata*, *Apis florea*, and *Apis mellifera*), soon after *Apis koschevnikovi*, *Apis andreniformis*, *Apis laboriosa*, and *Apis nigrocincta* reappear in the literature. Some 50% of all literature on Asian honeybees follows publication of Ruttner's classic work. Another major impetus for increased research on honeybees in Asia undoubtedly stems from the rather thorough cover given to this literature by Eva Crane and colleagues through some 50 odd years of Apicultural Abstracts.

Interestingly, the lion's share of work on Asian honeybees is also historically postcolonial in origin. It has also very largely resulted from the joint efforts of Asian and Western scientists working in tandem. On the Asian side, this year, 2010, also sees the 10th international conference of the Asian Apicultural Association, a body that has both stimulated Asian colleagues and made Western ones warmly received. Perusal of recent apicultural literature shows that East–West scientific alliances are increasing rapidly and bearing substantial fruit.

This volume is presented as a monograph. Monographs are usually understood to be complete and detailed expositions of a subject at an advanced level. While we believe that we have achieved this end through the inclusion of chapters by specialists in the field, it must be pointed out that while each chapter shows a reasonable depth of understanding, nonetheless they clearly indicate chasms in our knowledge of the honeybees of Asia. Much presented here is completely new and has, as yet, not been published in journals. Compared with the literature on western honeybees, that for Asia reveals very thin coverage for honeybee physiology, biochemistry, genetics, and pathology. This volume is a status quo report of what is known, and we fervently hope that this

collation will provide stimuli to broaden the base of the biology of the Asian honeybees.

Grahamstown, South Africa H.R. Hepburn
January 2011 S.E. Radloff

# Contents

# Contributors

**D.P. Abrol** Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu (J&K) 180 009, India, dharam_abrol@rediffmail.com

**Richard T. Corlett** Department of Biological Sciences, National University of Singapore, Singapore, Singapore 117543, corlett@nus.edu.sg

**R.M. Crewe** Social Insect Research Group, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa, robin.crewe@up.ac.za

**Orawan Duangphakdee** Ratchaburi Campus, King Mongkut's University of Technology, Thonburi, Bangkok 10140, Thailand, Orawan.dua@kmutt.ac.th

**Michael S. Engel** Division of Entomology, Natural History Museum, University of Kansas, 501 Crestline Drive-Suite #140, Lawrence, KS 66049-2811, USA, msengel@ku.edu

**Ingemar Fries** Department of Ecology, Swedish University of Agricultural Sciences, Uppsala 750 07, Sweden, ingemar.fries@ekol.slu.se

**Stefan Fuchs** Institut für Bienenkunde (Polytechnische Gesellschaft), FB Biowissenschaften, Goethe-Universität Frankfurt am Main, Karl von Frisch Weg 2 61440, Germany, s.fuchs@bio.uni-frankfurt.de

**H.R. Hepburn** Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa, r.hepburn@ru.ac.za

**Thomas Hoetzl** Institute of Zoology, University of Graz, Graz, Austria, frank.weihmann@uni-graz.at

**Gerald Kastberger** Institute of Zoology, University of Graz, Graz, Austria, gerald.kastberger@uni-graz.at

**Nikolaus Koeniger** Institut für Bienenkunde, (Polytechnische Gesellschaft) Fachbereich Biowissenschaften Goethe Universität, Frankfurt a.M. Karl-von-Frisch-Weg 2, 61440 Oberursel, Germany, nikolaus.koeniger@bio.uni-frankfurt.de

**Gudrun Koeniger** Institut für Bienenkunde (Polytechnische Gesellschaft), Fachbereich Biowissenschaften, Goethe Universität, Frankfurt a.M, Karl-von-Frisch-Weg 2, 61440, Oberursel, Germany, gudrun.koeniger@bio.uni-frankfurt.de

**Chariya Lekprayoon** Center of Excellence in Biodiversity, Department of Biology, Faculty of Sciences, Chulalongkorn University, Bangkok 10330, Thailand, Chariya.l@chula.ac.th

**Uma Partap** Honeybees Project, Sustainable Livelihoods and Poverty Reduction Programme, International Centre for Integrated Mountain Development (ICIMOD), PO Box 3226, Kathmandu, Nepal, upartap@icimod.org

**Mananya Phiancharoen** King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Bangkok 10140, Thailand, manaya.phi@kmutt.ac.th

**Christian W.W. Pirk** Social Insect Research Group, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa, cwwpirk@zoology.up.ac.za

**Sarah E. Radloff** Department of Statistics, Rhodes University, Grahamstown 6140, South Africa, s.radloff@ru.ac.za

**Deborah Smith** Department of Ecology and Evolutionary Biology/Entomology, University of Kansas, Haworth Hall, 1200 Sunnyside Ave, Lawrence, KS 66045, USA, debsmith@ku.edu

**Catherine L. Sole** Social Insect Research Group, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa, clsole@zoology.up.ac.za

**Ken Tan** Eastern Bee Research Institute of Yunnan, Agricultural University, Heilongtan, Kunming, Yunnan Province, People's Republic of China, eastbee@public.km.yn.cn

**Jürgen Tautz** BEE Group, Biozentrum, Universität Am Hubland, 97074 Würzburg, Germany, Tautz@biozentrum.uni-wuerzburg.de

**Natapot Warrit** Center of Excellence in Entomology, Department of Biology, Faculty of Sciences, Chulalongkorn University, Bangkok 10330, Thailand; Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA, Natapot.w@chula.ac.th

**Frank Weihmann** Institute of Zoology, University of Graz, Graz, Austria, frank.weihmann@uni-graz.at

**Ming-Xian Yang** Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa; Department of Zoology, Sichuan Agricultural University, Yaan, People's Republic of China, damoguyan2006@yahoo.com.cn

# Chapter 1
# The Asian Species of *Apis*

**Sarah E. Radloff, H.R. Hepburn, and Michael S. Engel**

## 1.1 Introduction

The number of species of honeybees recognised over the last two and a half centuries has varied quite considerably, following the original descriptions of *Apis mellifera* (1758) by Linnaeus and *Apis florea* (1787), *Apis cerana* (1793) and *Apis dorsata* (1793) by Fabricius. In the nineteenth century, Frederick Smith (1854–1871) described some 20 additional species, often based on single specimens; only his taxa *Apis andreniformis* (1858) and *Apis nigrocincta* (1861), however, survived in honeybee systematics. Contemporaneously, Gerstäcker (1863) published the first comprehensive phylogenetic and taxonomic treatise on *Apis,* and reduced all previously described forms (except *A. andreniformis* and *A. nigrocincta*, which he either missed or ignored) to only the original four Linnean and Fabrician species. Although Smith (1865) subsequently presented his case for seven species, the views of Gerstäcker (1863) prevailed into the twentieth century (Koschevnikov 1900–1905; Enderlein 1906; von Buttel-Reepen 1906).

Matters then rested for another half century, until Maa (1953) published an abstruse monograph in which he introduced some 24 species of honeybees within four genera. These taxa have subsequently been almost totally ignored in the apicultural literature, and the historically older views of Gerstäcker (1863) have endured until relatively recently. During the years leading up to the publication of Ruttner's (1988) monograph, a search for East Asian honeybees (probably stimulated by Maa's

S.E. Radloff
Department of Statistics, Rhodes University, Grahamstown 6140, South Africa
e-mail: s.radloff@ru.ac.za

H.R. Hepburn
Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa
e-mail: r.hepburn@ru.ac.za

M.S. Engel
Division of Entomology, Natural History Museum, University of Kansas, 501 Crestline Drive-Suite #140, Lawrence, KS 66049-2811, USA
e-mail: msengel@ku.edu

H.R. Hepburn and S.E. Radloff (eds.), *Honeybees of Asia*,
DOI 10.1007/978-3-642-16422-4_1, 

original paper) ensued, with *Apis laboriosa* re-announced (Sakagami et al. 1980), *A. andreniformis* re-established (Wu and Kuang 1986, 1987; Kuang 1983), *Apis koschevnikovi* rediscovered (Mathew and Mathew 1988; Rinderer 1988) and *A. nigrocincta* re-entering the scene (Hadisoesilo and Otis 1996). Finally, *Apis nuluensis* was described as a new species (Tingek et al. 1996). When Ruttner (1992) subsequently published his natural history of honeybees, he included *A. laboriosa*, *A. andreniformis* and *A. koschevnikovi* alongside the "traditional" four species. In the most recent taxonomy of honeybees, Engel (1999) applied a phylogenetic species concept and accordingly regarded *A. laboriosa* and *A. nuluensis* as synonyms of *A. dorsata* and *A. cerana*, respectively – a view that has not been widely accepted by apiculturists, who have tended to employ alternate species concepts (that is, either the biological species or the evolutionary species concepts). Even now, the number of recognised species of honeybees remains in a state of flux.

Conceptualisation of species recognition also changed through the centuries, from the Platonic concept, exemplified by Linnaeus, to the slow introduction of the idea of a biological species, developed by Poulton (1908), Rensch (1929) and Dobzhansky (1937) and subsequently widely promulgated by Huxley (1940) and Mayr (1942). Indeed, today there are as many concepts for species recognition as there are putative honeybee species, and the very system by which we recognise biological units in nature is fiercely debated (e.g., Wheeler and Meier 2000). Moreover, honeybee researchers have focussed almost exclusively on the oldest of the currently used species concepts, the biological species concept.

Nonetheless, whether a species is diagnosed by population phenomena (the biological species concept), evolutionary lineages (the evolutionary species concept) or genealogical descent (the phylogenetic species concept), classification still requires that species-specific characteristics be brought to bear in the circumscription of species. Likewise, there have been several phylogenetic analyses conducted (Deodikar 1960; Sakai et al. 1986; Sheppard and Berlocher 1989; Alexander 1991; Garnery et al. 1991; Smith 1991; Petrov 1992; Willis et al. 1992; Engel and Schultz 1997; Engel 1999; Raffiudin and Crozier 2007; cf. Chap. 2), all based implicitly on the correctness of the named species.

Following the non-Linnean views of DuPraw (1964), however, coupled with the idea that sub-specific categories are untenable in a contiguous population (Wilson and Brown 1951), Hepburn and Radloff attempted to bypass the problem of classification by designating statistically defined populations of honeybees under the new coinage of "morphoclusters" (Hepburn et al. 2001a, b, 2005; Radloff et al. 2005a, b, c, 2010). They have since accepted the arguments of Engel (personal communication) that "morphoclusters" are really statistically defined "subspecies" to which they had been inconsistently applying trinomial names. Here, we report the results of a full multivariate morphometric analysis of the Asian species of *Apis* and correct the classification of *Apis* in accordance with the rules of the International Code of Zoological Nomenclature.

The systematics of honeybees has also undergone a paradigm shift as earlier evolutionary taxonomic methods and systems of organisation have become passé, having been replaced by the contemporary emphasis on populations, the statistical

distribution of morphological characters and the reconstruction of evolutionary lineages. Moreover, there has been no diagnostic account of the Asian species of *Apis* since Maa (1953). Here, we present the analyses of the currently recognised species of *Apis*: *A. andreniformis*, *A. cerana*, *A. dorsata*, *A. florea*, *A. koschevnikovi*, *A. laboriosa*, *A. mellifera*, *A. nigrocincta* and *A. nuluensis* (noting that *laboriosa* and *nuluensis* are valid only under the antiquated biological species concept). We combine metrical and descriptive morphological characters, DNA characteristics (cf. Chap. 4), behaviour and nesting (cf. Chap. 6) so as to holistically define honeybee species and more easily identify them, either in an equipped laboratory or under field conditions.

## 1.2 The Dwarf Honeybees

### *1.2.1 Identification of* Apis andreniformis *and* Apis florea

The distinctness of both *A. florea* and *A. andreniformis* as unequivocal, valid biological species is now well established and rests on the cumulative knowledge of the morphology of drone genitalia (Lavrekhin 1935; Ruttner 1975, 1988; Kuang and Li 1985; Wu and Kuang 1986, 1987; Wongsiri et al. 1990; Chen 1993; Patinawin and Wongsiri 1993), differences in nest structure (Thakar and Tonapi 1962; Dung et al. 1996; Rinderer et al. 1996; cf. Chap. 6), chemical profiles of beeswax (Aichholz and Lorbeer 1999, 2000; cf. Chap. 6), morphometrics (Jayavasti and Wongsiri 1992; Rinderer et al. 1995), allozyme polymorphism (Nunamaker et al. 1984; Li et al. 1986; Gan et al. 1991), mtDNA sequence divergences (Smith 1991; Willis et al. 1992; Nanork et al. 2001; cf. Chap. 4), flight (Radloff et al. 2001; cf. Chap. 13), timing of mating flights (Rinderer et al. 1993; Otis et al. 2001; cf. Chap. 8), sexual selection (Baer 2005) and niche differences (Oldroyd et al. 1992; Booncham et al. 1995; Rinderer et al. 2002; cf. Chap. 6). Several of these differences contribute to the complete reproductive isolation between the two species (Koeniger and Koeniger 1991, 2000, 2001; Otis 1991; Dung et al. 1996; cf. Chap. 8).

Unfortunately, accurate identifications of the dwarf honeybees in the older literature are often difficult to assess because the worker bees are morphologically similar and the species are sympatric over a wide area that extends from north-eastern India to Indochina (Otis 1996; cf. Chap. 3). Some of the historical confusion between *A. florea* and *A. andreniformis* stems from the fact that their classification is based on workers, which do not show great morphological differentiation. Moreover, the descriptions and taxonomic keys of Maa (1953) were based on very limited numbers of specimens, and some of the purported differences between the two species become blurred if many workers of a colony are analysed.

The most reliable characteristics to rapidly distinguish *A. florea* and *A. andreniformis* are as follows: in drones, the “thumb” of the bifurcated basitarsus of the

hind leg, which in *A. florea* is much longer than that of *A. andreniformis* (Ruttner 1988); the structure of the endophallus (Lavrekhin 1935; Wongsiri et al. 1990; Koeniger 1991; cf. Chap. 8); the cubital index in worker bees, which, at about 3 in *A. florea*, is significantly less than that in *A. andreniformis*, which is at about 6; the jugal-vannal ratio of the hindwing, which, at about 75 in *A. florea* is greater than that of *A. andreniformis*, at about 65; the abdominal tergite 2, which in *A. andreniformis* is deeply punctate, unlike that in *A. florea*; and the marginal setae on the hind tibiae, which in *A. florea* are usually entirely white, while those in *A. andreniformis* are dark-brown to blackish, in sclerotised, non-callow individuals.

Several subspecies, varieties, and nationes of *A. florea,* first described by Fabricius (1787), have been described over the last two centuries (Engel 1999). *A. andreniformis* was described by Smith (1858) as a species distinct from *A. florea* (Fabricius 1787) but was usually included among the varieties or subspecies of the latter for nearly a century, until its re-establishment as a species by Maa (1953). Although *A. andreniformis* was often considered a subspecies of *A. florea*, no sub-specific taxa have ever been proposed for *A. andreniformis*. Unfortunately, an unspecifiable number of specimens of *A. andreniformis* may have been misidentified as *A. florea* during this period. All named forms were eventually resolved into colour variants from widely separated localities (Dover 1929). Subsequently, Maa (1953) synonymised all previous such taxa of earlier workers (Gerstäcker 1863; Enderlein 1906; von Buttel-Reepen 1906; Cockerell 1911; Dover 1929), and no sub-specific categories of *A. florea* have been proposed since then (Hepburn et al. 2005).

The mistaken notion that abdominal tergites 1 and 2 of *A. florea* are reddish and other segments at least partially reddish, while those of *A. andreniformis* are uniformly black, still permeates the literature. However, an inspection of several hundred workers from several different colonies of each species quickly demonstrates the extreme variation in pigmentation. This precludes these characters as a useful distinguishing trait – a point actually recognised rather long ago (Drory 1888; Dover 1929). Finally, the combs of the two species are very different (Rinderer et al. 1996; cf. Chap. 6). Full bibliographies of the literature on *A. florea* and *A. andreniformis* are given in Hepburn and Hepburn (2005, 2009), respectively; cf. Chap. 20).

### *1.2.2* Apis andreniformis *F. Smith (1858)*

*A. andreniformis*, the smallest of the honeybees, has been studied far less than *A. florea*. To date, there has been a single univariate morphometric comparison of *A. andreniformis* from southeastern Thailand and Palawan Island in the Philippines (Rinderer et al. 1995). These two widely separated populations (~3,000 km) differed only in a few characters that related to wing and metatarsal lengths, which indicates that it is likely a very homogeneous species. Likewise, estimates of the mtDNA haplotype divergence within the species was about 2% for *A. florea*

and 0.5% for *A. andreniformis*, indicating rather homogeneous populations in both cases (Smith 1991; cf. Chap. 4).

The only published multivariate morphometric analysis of this species is the recent study of Rattanawannee et al. (2008), who collected 67 colonies throughout Thailand – 30 of which were for morphometric analysis and the remaining 37 for DNA polymorphism. Twenty characters were used to assess morphometric variation. Principal component analysis yielded four factor scores, which, when plotted, formed a single group, supported by a dendrogram generated from the cluster analysis. Using linear regression analysis, Rattanawannee et al. (2008) demonstrated the clinal pattern of morphometric characters, wherein body size decreases from west to east, associated with decreasing altitude, while it increases from south to north, associated with increasing altitude. Genetic variation, however, based on the sequence analysis of the cytochrome oxidase subunit b, yielded two groups – a result taken as tentative, pending more extensive analyses across the whole area of distribution of *A. andreniformis* (cf. Chap. 3).

### *1.2.3* Apis florea *Fabricius (1787)*

Several univariate morphometric studies on regional or country bases have appeared through the years, but they have not affected the taxonomy of the species. In the first multivariate morphometric analysis of *A. florea*, Ruttner (1988) had only limited material, from geographically non-contiguous regions. Although the data were insufficient for a comprehensive analysis, Ruttner (1988) demonstrated geographic variability and obtained three morphoclusters for *A. florea*. Recently, Tahmasebi et al. (2002) analysed *A. florea* and defined two morphoclusters from a geographical continuum in Iran. Combining their data with that of Ruttner (1988) and Mogga and Ruttner (1988), they also reported three morphoclusters for all *A. florea*; but again, a lack of geographical contiguity applies to these data as well. A multivariate study of the *A. florea* of Thailand has also been conducted (Chaiyawong et al. 2004). The raw data of Ruttner (1988), Tahmasebi et al. (2002), Mogga and Ruttner (1988) and Chaiyawong et al. (2004) were included in a subsequent study in which previous gaps in the distribution had been filled, finally allowing a comprehensive morphometric database for *A. florea* over its entire distribution to be compiled (Hepburn et al. 2005).

Principal component, discriminant and cluster analyses using the single linkage (nearest neighbour) procedure were carried out and produced a dendrogram of three main clusters (Fig. 1.1). Phenetically, cluster 1 initially linked colonies from Myanmar and Thailand, followed by Cambodia and finally Northern Vietnam; cluster 2 initially linked colonies from Oman, North India and Nepal, followed by those from South India; cluster 3 linked colonies from Iran and Pakistan; while clusters 2 and 3 linked colonies from Southern Vietnam (Fig. 1.1).

Radloff and Hepburn (1998, 2000) and Hepburn et al. (2001b) established empirically that the greater the sampling distances between localities, the greater

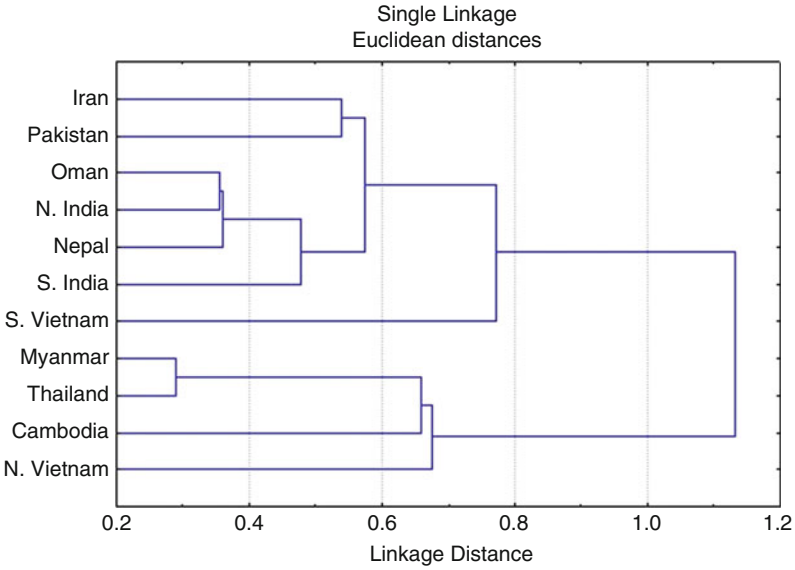


**Fig. 1.1** Hierarchical clustering dendrogram for *Apis florea*, derived from single linkage clustering on morphometric characters: length of femur (5); length of tibia (6); length of metatarsus (7); tergite 3; longitudinal (9); tergite 4; longitudinal (10); length of forewing (17); wing angle G18 (25), averaged for countries. The original coded numbers assigned to these characters by Ruttner (1988)

the likelihood that artefactual morphoclusters would emerge in multivariate analyses. Conversely, where between-group variation is larger than within-group variation, biometric subgroups falling within smaller geographic domains may be swamped and obscured. Radloff et al. (2003b) also established the statistical significance of both colony sample size and individual bee sample size to studies of honeybee populations. These principles are particularly useful in the analyses of previous studies of *A. florea* and explain why Tahmasebi et al. (2002) defined two morphoclusters when they analysed the *A. florea* of Iran. Combining their data with that of Ruttner (1988) and Mogga and Ruttner (1988), Radloff et al. (2003b) reported three morphoclusters. In both studies, however, there was still a lack of geographical contiguity in the samples and each of the three groups was separated by intervals of about 3,000 km. When Hepburn et al. (2005) analysed the bees from the whole spectrum of localities sampled, the clinal nature of the morphometric measurements of the species became readily apparent. Precisely this same pattern was obtained in studies of *A. cerana* (Radloff et al. 2010).

On a mesoscale level, there have been several regional studies of morphometric variation in *A. florea* in India and Iran, representing sampling intervals of about 3,000 km. In northwestern India and eastern Pakistan, extending along a north–south transect between 25° and 32°N latitude, a transition in the populations occurs. There are significant interlocality differences in both the mean values of morphometric characters and their coefficients of variation, for most

characters measured (Narayanan et al. 1960; Bhandari 1983; Sharma 1983) – implying heterogeneity in the population. Likewise, at hotter, drier and lower latitudes, *A. florea* are smaller than those at cooler and higher latitudes, leading to the proposition of possibly different ecotypes associated with climate at particular latitudes (Narayanan et al. 1960; Bhandari 1983). There are, however, alternative views on this point (Sharma 1983). Within a sample from India, Hepburn et al. (2005) obtained a strong, significant positive correlation between altitude and the principal component variables that reflect size. This pattern might benefit from additional attention.

Tahmasebi et al. (2002) reported an analysis of *A. florea* from 26 localities in Iran and obtained two morphoclusters: a western group of larger bees at higher latitudes (29–34°) and a lower latitude group of smaller bees to the east (<29° latitude). In the study of Hepburn et al. (2005), one morphocluster with two indistinct clusters of smaller eastern and larger western bees were noted. Here, the distributional variation in morphometric characters is clinal: northwestern bees are larger than southeastern ones (Özkani et al. 2009). In the final analysis, *A. florea* is a single species comprised of three discernible morphoclusters. The northwestern-most bees comprise a morphocluster that is statistically quite distinct from that to the southeast; but they are not isolated. Rather, they are joined by large areas of intermediate forms, resulting in a continuous cline in morphometric traits within this panmictic species.

## 1.3 The Medium-Sized Bees

### *1.3.1 Identification of* Apis cerana, Apis koschevnikovi, Apis nigrocincta *and* Apis nuluensis

The sympatric occurrence of *A. cerana* with other medium-sized bees, *A. koschevnikovi*, *A. nigrocincta* and *A. nuluensis*, in southeastern Asia, unfortunately means that an indeterminable amount of previous "*A. cerana*" literature may inadvertently include data derived from other species (Hepburn et al. 2001a). To assist in overcoming this problem, we list metric characters that, in combination, separate these four species as follows: firstly, the cubital indexes of the forewings, which are 3.9 for *A. cerana*, 7.2 for *A. koschevnikovi*, 3.7 for *A. nigrocincta* and 2.4 for *A. nuluensis* – quickly separating paired comparisons for all, with the exception of an *A. cerana* and *A. nigrocincta* option. To separate this combination (*A. cerana* from *A. nigrocincta*), three measurements may be used: the length of the basal portion of the radial cell of the forewing, which is 1.2 mm in *A. cerana* and 1.8 mm in *A. nigrocincta*; the length of the apical portion of the radial cell, which is 1.8 mm in *A. cerana* and 1.1 mm in *A. nigrocincta*; and the length of the labial palp, which is 1.8 mm in *A. cerana* and 3.7 mm in *A. nigrocincta*.

### 1.3.2 Apis cerana *Fabricius (1793)*

Over the last two decades, great strides have been made following Ruttner's (1988) first multivariate analysis of this species. Subsequent authors used Ruttner's interpretations of *A. cerana* as a new baseline and concentrated on morphoclusters derived from multivariate analyses on a microscale level (Muzaffar and Ahmad 1989; Pesenko et al. 1989; Rinderer et al. 1989; Otis and Hadisoesilo 1990; Singh et al. 1990; Sulistianto 1990; Szabo 1990; Ono 1992; Verma 1992; Verma et al. 1994; Hadisoesilo and Otis 1996; Fuchs et al. 1996; Damus and Otis 1997; Sylvester et al. 1998) as well as on a more regional, mesoscale level (Yang 1986, 2001; Peng et al. 1989; Diniz-Filho et al. 1993; Damus and Otis 1997; Tilde et al. 2000; Hepburn et al. 2001a, b; Kuang 2002; Radloff and Hepburn 2002; Smith 2002; Tan et al. 2003; Radloff et al. 2003a, 2005a, b, c).

Historically, unravelling the structural complexity of *A. cerana* (Fabricius 1793) has been a continuous process, the details of which were recently given by Radloff et al. (2010). They reported the first multivariate morphometric analysis of *A. cerana* across its full geographical range and identify the statistically definable morphoclusters and subcluster populations within them. Principal component (PC) plots, using both the first and second PC scores and the first and third PC scores, did not reveal distinct morphoclusters. However, a substructuring of the PC plots was obtained by introducing local labelling and running a hierarchical cluster analysis, using the mean scores for PC 1 to 3 to identify homogeneous morphoclusters. This approach revealed six main morphoclusters, which were defined (Radloff et al. 2010) as follows (cf. Fig. 3.3):

1. Morphocluster I, "Northern *cerana*", which extends from northern Afghanistan and Pakistan through northwest India, across southern Tibet, northern Myanmar, China and northeasterly into Korea, far eastern Russia and Japan. Six subclusters or populations are morphometrically discernible within this morphocluster (a) an "Indus" group in Afghanistan, Pakistan and Kashmir; (b) a "Himachali" group in Himachal Pradesh, India; (c) an "Aba" group in Ganshu and Sichuan provinces in China, northern China and Russia; (d) a subcluster in central and eastern China; (e) a "southern *cerana*" subcluster in southern Yunnan, Guangdong, Guangxi and Hainan in China and (f) a "*japonica*" group in Japan and Korea.
2. Morphocluster II, "Himalayan *cerana*", which includes the bees of northern India and some of southern Tibet and Nepal. Two subclusters are discernible within this morphocluster: the bees of the northwest, which are termed the "Hills" group, and those of the northeast, termed the "Ganges" group (cf. Figs. 3.1 and 3.3).
3. Morphocluster III, "Indian plains *cerana*", which occurs across the plains of central and southern India and Sri Lanka as a fairly uniform population, long known as "plains *cerana*" in this subcontinent (cf. Figs. 3.1 and 3.3).
4. Morphocluster IV, "Indo-Chinese *cerana*", which forms a compact group in Myanmar, northern Thailand, Laos, Cambodia and southern Vietnam (cf. Figs. 3.1 and 3.3).

5. Morphocluster V, "Philippine *cerana*", which is restricted to the Philippines, but with the exclusion of most of Palawan Island, which instead groups with morphocluster VI. Within these islands, there are subclusters, and these bees are termed after the major island groups located there: "Luzon" bees, "Mindanao" bees and "Visayas" bees. The latter two subclusters show closer morphometric similarity than the former (cf. Figs. 3.1 and 3.3).
6. Morphocluster VI, "Indo-Malayan *cerana*", which extends from southern Thailand, through Malaysia and Indonesia. This large area consists of a rather morphometrically uniform bee, below the South China Sea. Three subclusters are discernible within this morphocluster: (a) Palawan (Philippines) and Borneo bees; (b) Malay Peninsula, Sumatera and some Sulawesi bees; and (c) Indonesia (Java, Bali, Irian Jaya, some Sulawesi and Sumatera) bees (cf. Figs. 3.1 and 3.3).

We must now consider how these results relate to earlier geographically large-scale analyses. When all of the mesoscale morphoclusters of Radloff et al. (2010) are compared with the new macroscale results, the only discrepancies are that, in the former, (1) the bees of the Philippines were included with those of Indonesia and Borneo; and (2) the bees of Japan are now placed in the Northern Asia morphocluster of the latter. However, there are differences between the mapped morphocluster results of Ruttner (1988) and Damus and Otis (1997) and those of Radloff et al. (2010). These discrepancies are best explained by the sampling differences in each study, which affected the degree of morphometric discrimination of the honeybees of Japan.

Ruttner (1988) had access to only a very small sample of large *A. cerana* from China and none from Russia. The only morphocluster I bees available to him were from the far northwest of the *A. cerana* range (Afghanistan and Pakistan) and some 6,000 km distant from Japan – the bees of which form a subcluster in a continuum of *A. cerana* morphocluster I. Gaps in the sampling inevitably resulted in the differences between Afghani and Japanese *A. cerana* being artefactually magnified. The dataset of Damus and Otis (1997) was based on the much smaller bees of the more southerly oceanic islands (Philippines, Indonesia, Borneo, etc.) with the same effect.

Returning to the matter of sampling, many thorough multivariate studies of *A. cerana*, sampled at a microscale basis, had been published; but, with the advantage of hindsight, the effects of limited sampling are evident. An important series of papers was published on sub-Himalayan *A. cerana*; however, the areas sampled were widely separated, and the net result was discrimination of seven distinct morphoclusters (Singh et al. 1990; Verma 1992; Verma et al. 1994). When the original data from all these papers were subsequently combined into a much larger dataset in collaboration with those authors, and for which the previous geographical gaps were filled, the newer multivariate analysis (now on a geographical continuum in the sub-Himalayan region) yielded only four morphoclusters for the same region – two of which contained biometric subclusters (Hepburn et al. 2001b).

Analysis of the *A. cerana* of the western sub-Himalayas yielded an additional Hindhu Kush morphocluster, bringing the Himalayan string of morphoclusters to

five (Radloff et al. 2005a). The analysis found that high variance domains occur at the edges of the morphoclusters and biometric subclusters. The bees decrease in size from west to east, but increase in size with increasing altitude. When analyses were subsequently extended from Afghanistan to Vietnam, covering all of southern-mainland Asia, scores from the principal components analysis yielded five statistically identifiable morphoclusters (Radloff et al. 2005b). At this continental resolution, the five morphoclusters previously obtained in the regional analyses of the Himalayan string (Hepburn et al. 2001b; Radloff et al. 2005a) were reduced to three, which were also coherently distributed with the different climatic zones of the region (Radloff et al. 2005b).

In a parallel series of studies on the *A. cerana* of China, Tan et al. (2002, 2003) showed that bees from the northern high-altitude areas of Yunnan Province were clearly larger and darker and showed similarities to samples from Beijing, Nepal and northern India, whereas bees from southern Yunnan clustered with the bees of Thailand and Vietnam. These results were completely consistent with those of Radloff et al. (2005b) for the bees of southern Yunnan. Morphometric analyses of *A. cerana* from oceanic Asia yielded two distinct morphoclusters, bringing the then total number of morphoclusters to seven (Radloff et al. 2005c). On completion of the above series of regional mesoscale studies, the newly formed comprehensive dataset for all *A. cerana* was subjected to multivariate morphometric analysis. The final result was that six distinct morphoclusters of *A. cerana* were obtained, as discussed above (Radloff et al. 2010; cf. Fig. 3.3).

### *1.3.3* Apis koschevnikovi *Enderlein (1906)*

*A. koschevnikovi* was originally described by Enderlein (1906) as "*Apis indica* variety *koschevnikovi*" and by von Buttel-Reepen (1906) as "*Apis mellifica indica* variety *koschevnikovi*". Authorship for this species has however been formally assigned to Enderlein (Engel 1999) as *A. koschevnikovi* Enderlein (1906), in accordance with nomenclatural practice. With few exceptions (Maa 1953; Goetze 1964), there were no accounts of *A. koschevnikovi* until its rediscovery eight decades later in Borneo (Mathew and Mathew 1988; Rinderer 1988; Tingek et al. 1988). However, *A. koschevnikovi* had indeed been widely collected in the Sundaland region of Southeast Asia during the interim, as evidenced by collections in various museums (Otis 1996). In a recent flurry of publications (Hepburn and Hepburn 2008), it has been established that *A. koschevnikovi* is a morphometrically distinct species (Tingek et al. 1988; Rinderer et al. 1989; Ruttner et al. 1989; Sulistianto 1990; Hadisoesilo et al. 1999), reproductively isolated (Koeniger et al. 1996c) and differing in both nuclear and mitochondrial DNA regions (Arias et al. 1996; Takahashi et al. 2002; Raffiudin and Crozier 2007) from other species of *Apis*, with which it has a sympatric distribution.

Although most characters of length are some 10–15% greater in worker honeybees of *A. koschevnikovi* than in *A. cerana* (Rinderer et al. 1989; Sulistianto 1990),

these species may be confused in alcohol-preserved specimens that do not show the natural reddish-yellow brightness of the former. Multivariate analyses of *A. koschevnikovi* samples from Malaysia, Borneo and Indonesia clearly established that this species is comprised of a single morphocluster (Hadisoesilo et al. 2008). Moreover, the morphocluster can be delimited with as few as 12 morphological characters. It would also appear to be a very homogeneous species, in comparison with *A. cerana,* over the same area of distribution, because the average coefficient of variation in *A. koschevnikovi* is 1.8%, while in *A. cerana*, it is 4.3% for the same characters (Hadisoesilo et al. 2008).

### *1.3.4* Apis nigrocincta *F. Smith (1861)*

The life history of *A. nigrocincta* F. Smith (1861) is curiously similar to that of *A. koschevnikovi*. Described as a new species by F. Smith (1861), it remained virtually unreported, with a few exceptions, for more than a century, until it was re-examined in the 1990s. In the first instance, Hadisoesilo et al. (1995) detected two distinct groups of honeybees in Sulawesi, Indonesia. A discriminant analysis of these bees showed one group to be *A. cerana* and the other as neither *A. cerana* nor *A. koschevnikovi*. Moreover, these then unidentified bees appeared similar to *A. nigrocincta* when compared to the holotype. In rapid succession, the Guelph group confirmed that the unknown bees were indeed *A. nigrocincta* and that they occur in the Philippines as well (Damus and Otis 1997). Further multivariate analyses confirmed that *A. nigrocincta* occurred in western Sulawesi, Mindanao Island in the Philippine chain and on Sangihe Island, situated between the two (Damus and Otis 1997).

Studies of drone flight times further supported the status of *A. nigrocincta* as a species distinct from *A. cerana* (Hadisoesilo and Otis 1996; Otis et al. 2001). Interestingly, they found no differences in the drone genitalia of *A. nigrocincta* and *A. cerana*. The reality of *A. nigrocincta* as a valid species continued to grow when it was shown that the cappings of drone cells in *A. nigrocincta* lacked the well-known pore that is present in *A. cerana* (Hadisoesilo and Otis 1998). Jayavasti and Wongsiri (1992) were able to differentiate *A. nigrocincta* and *A. cerana* on the basis of sting morphology, while Keeling et al. (2001) established species-specific differences in the mandibular gland pheromones of queens. The species was also recognised in taxonomic studies of *Apis* by Engel (1999).

Shortly afterwards, the separation of these species through mtDNA analyses (Smith et al. 2000), receptor gene sequences (Raffiudin and Crozier 2007) as well as new haplotypes for the non-coding region of mtDNA (Takahashi et al. 2002) confirmed the *A. nigrocincta* species. More recent analyses of nuclear and mitochondrial DNA sequences further support the validity of *A. nigrocincta* (Arias and Sheppard 2005). Finally, Raffiudin and Crozier (2007) supported *A. nigrocincta* as a valid species on the basis of general biology, DNA, acoustics, waggle dance and combs.

Only in the last decade have we acquired sufficient evidence to consider *A. nigrocincta* as a reasonably well-defined valid species. Hadisoesilo and Otis (1996) and Otis et al. (2001) demonstrated that, although sympatric with *A. cerana*, *A. nigrocincta* is reproductively isolated from other Asian *Apis* species in the timing of its mating flights, is distinguishable from other *Apis* species in morphometric analyses (Hadisoesilo et al. 1995; Hadisoesilo and Otis 1996) and differs in mtDNA haplotypes (Smith et al. 2000, 2003). However, until very recently, its known distribution was limited to Indonesia and the Philippines (Otis 1996). Interestingly, Otis (1996) suggested that *A. nigrocincta* might have been derived from China, because it shares closer similarities with *A. cerana* from the mainland than from the southwest.

### *1.3.5* Apis nuluensis *Tingek et al. (1996)*

Just over a decade ago, Tingek et al. (1996) collected bees at flowers on Gunung Emas at an altitude of about 2,000 m, which appeared distinctly different from *A. cerana* and *A. koschevnikovi*. They conducted morphometric measurements on these blackish bees, using most of Ruttner's (1988) characters, and showed that they differed significantly from *A. cerana* and *A. koschevnikovi* workers and drones (with which they are sympatric), and accordingly described these bees as a new species, *A. nuluensis*. More extensive measurements were reported by Fuchs et al. (1996), who found that, in a principal component analysis plotting the first three of the axes derived from principal components, *A. nuluensis* was clearly separated from the other sympatric Asian *Apis* species. Moreover, a hierarchic cluster analysis of group centroids in canonical function space clearly showed that *A. nuluensis* is quite distinctly separated from the other species.

While the above remarks are restricted to inferences based entirely on morphometrics, other biological observations were soon brought to bear on the legitimacy of *A. nuluensis* as a distinct species under the biological species concept. Koeniger et al. (1996a, b) observed that the drone mating flight period was temporally completely isolated from those of *A. cerana* and *A. koschevnikovi*. Although there is a very small window of temporal overlap between *A. nuluensis* and *A. cerana,* the physical differences between the two would be adequate to obviate any heterospecific mating. This separation in time is a pre-mating barrier that provides complete reproductive isolation among the honeybees with which it is sympatric (Koeniger et al. 1996a, b; cf. Chap. 8).

Although *A. nuluensis* was initially proposed on the basis of morphological and behavioural characters, Arias et al. (1996) analysed variable sites for the ND2 mitochondrial gene as well as for the intron of EF-1α – the results of which indicate that *A. nuluensis* and *A. cerana* are closely related or even that the former was derived from the latter, which challenges the validity of the species under more modern species concepts, such as the phylogenetic species concept. They concluded that *A. nuluensis* diverged from *A. cerana* more recently than did

*A. koschevnikovi*. Using a slightly different approach, Takahashi et al. (2002) and Tanaka et al. (2001) investigated the haplotypes for the non-coding region of mitochondrial DNA and reached essentially the same conclusion as Arias et al. (1996). Similarly, on the basis of morphometrics, Fuchs et al. (1996) concluded that *A. nuluensis* shares a greater similarity with *A. cerana* than with *A. koschevnikovi*. *A. nuluensis* is thus far known only from montane forests on the Gunung Emas in Sabah State, Malaysian Borneo. This area is at the northeastern tip of mountain ranges that extend continuously for about 1,000 km to the southwest, along a spine of mountains that extends two-thirds the length of Borneo. The region is remote, sparsely inhabited and not readily accessible. It seems highly likely that *A. nuluensis* occurs along this spine.

## 1.4 The Giant Honeybees

### *1.4.1* Apis dorsata *Fabricius (1793)*

The classification of the giant honeybees, *A. dorsata* and *A. laboriosa,* has long been problematical. The former was described by Fabricius in 1793 and various forms were introduced between then and the time of Maa (1953). Maa recorded the various synonymies that had previously arisen and then reshaped and split the species into *A. breviligula* (one specimen from the Philippines), *A. binghami* (Sulawesi, formerly the Celebes) and *A. dorsata* (the wider distribution as known today). Over the next three decades, however, none of the names proposed by Maa (1953) appeared in the apicultural literature in any form other than "*A. dorsata*".

The next important discussion of these bees was that of Ruttner (1988), who noted that the standard deviations of several morphometric characters, representing widely separated localities were very small indeed so that *A. dorsata* appeared very homogeneous. He further argued that differences regarded by some as species-specific in the *A. dorsata* group are of the same order of magnitude as those used to discriminate subspecies of *A. mellifera*. Acknowledging some unusual aspects of the biology of *A. laboriosa*, Ruttner (1988) nonetheless was not prepared to recognise this bee as a clear-cut species, especially in the light of the report that no differences could be found in the male genitalia of *A. dorsata* and what purported to be "*A. laboriosa*" (McEvoy and Underwood 1988). He did, however, support the subspecies of *A. d. binghami*, *A. d. breviligula* and *A. d. dorsata*. *A. d. breviligula* is a conspicuously short-tongued bee of the Philippines, whose behaviour differs in important respects from *A. d. dorsata*. Congregations of several nests, common in areas of the latter, do not occur in those of the former; likewise, seasonal migration, also common in the former, is absent from the latter (Morse and Laigo 1968). *A. d. binghami* is a long-tongued, long-winged form, also isolated at the periphery of *A. dorsata* distribution in Sulawesi. Whether peripheral isolates should be considered as taxonomically distinct is a matter that is open to debate (Lo et al. 2010).

The history of works on *A. dorsata* once again illuminates the problem of sample size. When the spectrum of sampling has been wide, even though it contains many geographical gaps, it may be that a species seems rather homogeneous; and so it appeared to Ruttner. Prior to Ruttner (1988), however, many smallish and preliminary investigations had been reported. Most such studies emanated from India (Ratnam 1939; Deodikar 1959a, b, Deodikar et al. 1977; Trehan and Singh 1961; Jain 1967; Kshirsagar 1969; Sharma 1983; Bhandari 1983; Mujumdar and Kshirsagar 1986; Singh et al. 1990) and revolved around populations of northwest India, where great variations in altitude occur. All of these studies on the morphometrics and population structure of *A. dorsata* demonstrated that the populations sampled showed significant interlocality variation, which attests to the heterogeneity of these bees. Similar results were reported elsewhere (Kuang 1986). Unfortunately, there has not been any comprehensive multivariate morphometric analysis over the entire range of *A. dorsata* to date. However, it may well eventuate that the inferences from nuclear and mitochondrial DNA sequence data will prove more informative than those derived from morphometrics (Arias and Sheppard 2005; cf. Chap. 4).

In any event, over the last century, there have been only three "pre-biological species" taxonomic systematists (Enderlein 1906; von Buttel-Reepen 1906; Maa 1953) and two post-Huxley systematists (Daly 1985; Engel 1999) within honeybee systematics. Engel (1999) is the only contemporary systematist working on honeybees who presents both usage views: one in which there exists only *A. dorsata* and the other in which there exist *A. d. binghami*, *A. d. breviligula* and *A. d. dorsata*. The practice among honeybee biologists has, however, been to use the trinomial epithet as a tool on which to simply apply their names, based on inferences about the magnitude of differences they encounter. In these circumstances, the post-Ruttner apicultural literature abounds with the names *A. d. binghami*, *A. d. breviligula* and *A. d. dorsata*, as well as *A. laboriosa*. More recently, the names *A. binghami*, *A. breviligula* and *A. dorsata*, as well as *A. laboriosa*, are beginning to appear in common usage within the literature, which may reflect a growing consensus on the matter under certain species concepts (Lo et al. 2010). It would appear that total evidence and quantitative approaches, uniting multiple, independent lines of evidence, will be needed in place of morphometrics in the circumscription of species for this particular group of bees.

### *1.4.2* Apis laboriosa *F. Smith (1871)*

Like other lesser-known species of honeybees, the Himalayan *A. laboriosa* remained virtually unreported for a century after its original description by F. Smith (1871). While von Buttel-Reepen (1906) listed it as a subspecies of *A. dorsata*, Maa (1953) effectively resurrected its species status. More recently, Engel (1999) referred to *A. laboriosa* somewhat equivocally as *A. dorsata laboriosa* but did not accord it species status when applying a phylogenetic species concept.

Under both the biological and evolutionary species concepts, this form is considered a valid species and a recognised taxonomical entity. Real interest in *A. laboriosa* gained momentum following a major morphometric and biogeographical analysis by Sakagami et al. (1980). They established unequivocally that it was different from *A. dorsata* in 96 of 103 different morphometric measurements but, surprisingly, remained somewhat equivocal as to its taxonomical status. Li (1984), Chen (1993) and Trung et al. (1996) also distinguished the two species morphologically. McEvoy and Underwood (1988) argued, somewhat tenuously, that *A. laboriosa* and *A. dorsata* are sound species, based on the fact that no morphologically intermediate forms were known. These two species are very rarely sympatric, with *A. dorsata* usually occurring below altitudes of 1,500 m and *A. laboriosa* between altitudes of 2,500 and 4,000 m (Roubik et al. 1985; Allen 1995; Otis 1996; Thapa et al. 2001).

Nonetheless, a general consensus that *A. laboriosa* is a well-defined species under the biological species concept, developed only after (1) Li et al. (1986) and Kuang and Li (1988) clearly separated *A. laboriosa* from *A. dorsata* and other *Apis* species by their esterase isozyme profiles; (2) Underwood (1990) showed that *A. laboriosa* and *A. dorsata* are reproductively separated by drone mating flight times; (3) Blum et al. (2000) reported that no common chemical constituents were found in analyses of the cephalic and abdominal secretions of *A. laboriosa* and *A. dorsata*; (4) Aichholz and Lorbeer (1999, 2000) showed that the chemical profile of *A. laboriosa* beeswax differs unequivocally from that of all other *Apis*; (5) Kirchner et al. (1996) showed that, unlike *A. dorsata*, there is no acoustic component of the waggle dance in *A. laboriosa* and (6) Woyke et al. (2008) identified their differences in defensive behaviour. Sequence divergence between *A. laboriosa* and *A. dorsata* was consistent with behavioural data and supports the species status of *A. laboriosa* under the biological species concept (cf. Chap. 4).

## 1.5 Conclusion

Phylogenetic analyses strongly supported the basic topology that is recoverable from morphometric analysis, which groups the honeybees into three major clusters: giant bees (*A. dorsata*, *A. binghami* and *A. laboriosa*), dwarf bees (*A. andreniformis* and *A. florea*) and cavity-nesting bees (*A. mellifera*, *A. cerana*, *A. koschevnikovi*, *A. nuluensis* and *A. nigrocincta*). The clade of Asian cavity-nesting bees, however, included paraphyletic taxa. Exemplars of *A. cerana* collected from divergent portions of its range were less related to each other than were the sympatric taxa, *A. cerana*, *A. nuluensis* and *A. nigrocincta*. Nucleotide sequence divergence between allopatrically distributed western (*A. mellifera*) and eastern (*A. cerana*, *A. koschevnikovi*, *A. nigrocincta* and *A. nuluensis*) cavity-nesting species (being around 18% for the mitochondrial gene and 10–15% for the nuclear intron) suggested an earlier divergence for these groups than previously estimated from both morphometric and behavioural studies.

This latter finding necessitates a re-evaluation of the hypothesised origin of extant European, African and West Asian *A. mellifera*. In addition, the growing evidence of honeybee diversity in the geological past is not only expanding the total number of species but also forcing a reconsideration of global *Apis* biogeography. By example, the recent discovery of fossil honeybees in North America expands the lineage natively into the New World (Engel et al. 2009). The discovery of giant honeybees in Japan during the Miocene, demonstrates how, under changing climates, lineages considerably expanded their historical ranges (Engel 2006). Perhaps most interestingly, the diversity of basal fossil species currently suggests a more western origin for the honeybees, with a subsequent invasion and rapid radiation across Asia, which resulted in the remarkable array of species and challenging forms we see today.

## References

Aichholz R, Lorbeer E (1999) Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry. I. High-temperature gas chromatography. J Chromatogr A 855:601–615

Aichholz R, Lorbeer E (2000) Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry. II. High-temperature gas chromatography-chemical ionization mass spectrometry. J Chromatogr A 883:75–88

Alexander BA (1991) Phylogenetic analysis of the genus *Apis* (Hymenoptera: Apidae). Ann Entomol Soc Am 84:137–149

Allen MF (1995) Bees and beekeeping in Nepal. Bee World 76:185–194

Arias MC, Sheppard WS (2005) Phylogenetic relationships of honey bees (Hymenoptera: Apinae; Apini) inferred from nuclear and mitochondrial DNA sequence data. Mol Phylogenet Evol 37:25–35

Arias MC, Tingek S, Kelitu A, Sheppard WS (1996) *Apis nuluensis* Tingek, Koeniger and Koeniger, 1996 and its genetic relationship with sympatric species inferred from DNA sequences. Apidologie 27:415–422

Baer B (2005) Sexual selection in *Apis* bees. Apidologie 36:187–200

Bhandari VC (1983) Biometrical studies on *Apis florea* F and *Apis dorsata* F of northwestern India. Thesis, Himachal Pradesh University, Simla

Blum MS, Fales HM, Morse RA, Underwood BA (2000) Chemical characters of two related species of giant honeybees (*Apis dorsata* and *A. laboriosa*): possible ecological significance. J Chem Ecol 26:801–807

Booncham U, Wongsiri S, Thirakupt K (1995) Niche differences between *A. florea* and *A. andreniformis* in dry-evergreen forest, Thailand. In: International Conference on Tropical Bees Environment, Pedu Lake, Kedah, p 33

Chaiyawong T, Deowanish S, Wongsiri S, Sylvester HA, Rinderer TE, de Guzman L (2004) Multivariate morphometric study of *Apis florea* in Thailand. J Apic Res 43:123–127

Chen YC (1993) Apiculture in China. Agricultural Scitech Publishing House of China, Beijing

Cockerell TDA (1911) Descriptions and records of bees XXXV. Ann Mag Nat Hist 8:310–319

Daly HV (1985) Insect morphometrics. Annu Rev Entomol 30:415–438

Damus MS, Otis GW (1997) A morphometric analysis of *Apis cerana* F. and *Apis nigrocincta* Smith populations from Southeast Asia. Apidologie 28:309–323

Deodikar GB (1959a) Geographical distribution based on morphological characters (structure of genitalia and crossability among different bee species). Indian Bee J 23:60–61

Deodikar GB (1959b) Some taxonomic problems in honeybees. 1. The concept of supra-generic grouping. Bee World 40:121–124
Deodikar GB (1960) Some indications on the phylogenetic interrelations among bees. Bee Genet Inf Bull 1:15–16
Deodikar GB, Ghatge AL, Phadke RP, Mahindre DB, Kshirsagar K, Muvel KS, Thakar CV (1977) Nesting behaviour of Indian honeybees. III. Nesting behaviour of *Apis dorsata* Fab. Indian Bee J 39:1–12
Diniz-Filho JAF, Malapsina O, Pignata MIB (1993) Geographic variation in *Apis cerana indica* F.: a spatial autocorrelation analysis of morphometric patterns. J Apic Res 32:65–72
Dobzhansky TG (1937) Genetics and the origin of species. Columbia University Press, New York
Dover C (1929) Wasps and bees in the Raffles Museum, Singapore. Bull Raffles' Museum, Singapore 2:43–70
Drory E (1888) From my notebook: notes on bees from a trip around the world. Bienenvater 20:20–30 [in German]
Dung PX, Thai PH, Trung LQ (1996) Some comparative characteristics between *Apis florea* and *Apis andreniformis* in Minh Hai Province. In: Proceedings of the 3rd Asian apiculture association, Hanoi, p 62
DuPraw EJ (1964) Non-Linnean taxonomy. Nature 202:849–852
Enderlein G (1906) New honeybees and contribution to the distribution of the genus *Apis*. Stett Entomol Ztg 67:331–334 [in German]
Engel MS (1999) The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae; *Apis*). J Hymenopt Res 8:165–196
Engel MS (2006) A giant honey bee from the Middle Miocene of Japan (Hymenoptera: Apidae). Am Mus Novit 3504:1–12
Engel MS, Schultz TR (1997) Phylogeny and behavior in honey bees (Hymenoptera: Apidae). Ann Entomol Soc Am 90:43–53
Engel MS, Hinojosa-Diaz IA, Rasnitsyn AP (2009) A honey bee from the Miocene of Nevada and the biogeography of *Apis* (Hymenoptera: Apidae; Apini). Proc Calif Acad Sci 60: 23–38
Fabricius JC (1787) Mantissa insectorum. Hafniae, Proft [in Latin, cited from Maa 1953]
Fabricius JC (1793) Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus. Hafniae, Proft [in Latin, cited from Maa 1953]
Fuchs S, Koeniger N, Tingek S (1996) The morphometric position of *Apis nuluensis* Tingek, Koeniger and Koeniger, 1996 within cavity-nesting honeybees. Apidologie 27:397–405
Gan YY, Otis GW, Mardan MB, Tan SG (1991) Allozyme diversity in Asian *Apis*. In: Smith DR (ed) Diversity in the genus *Apis*. Westview, Boulder, pp 117–130
Garnery L, Vautrin D, Cornuet JM, Solignac M (1991) Phylogenetic relationships in the genus *Apis* inferred from mitochondrial DNA sequence data. Apidologie 22:87–92
Gerstäcker CEA (1863) On the geographical distribution and varieties of the honeybee, with remarks upon the exotic honeybees of the old world. Ann Mag Nat Hist 11(270–283): 333–347
Goetze GKL (1964) The honeybee under natural and beekeeping conditions. Part 1. Systematics, reproduction and heritability. Monogr Angew Entomol 19:1–20 [in German]
Hadisoesilo S, Otis GW (1996) Drone flight times confirm the species status of *Apis nigrocincta* Smith, 1861 to be a species distinct from *Apis cerana* F., 1793, in Sulawesi, Indonesia. Apidologie 27:361–369
Hadisoesilo S, Otis GW (1998) Differences in drone cappings of *Apis cerana* and *Apis nigrocincta*. J Apic Res 37:11–15
Hadisoesilo S, Otis GW, Meixner M (1995) Two distinct populations of cavity-nesting honeybees (Hymenoptera: Apidae) in South Sulawesi, Indonesia. J Kans Entomol Soc 68:399–407
Hadisoesilo S, Meixner M, Ruttner F (1999) Geographic variation within *Apis koschevnikovi* Buttel-Reepen, 1906, in Borneo. Treubia 31:299–306