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Geographical and population diversity of African honeybees, *Apismellifera* L. (Hymenoptera: Apidae): A review

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ABSTRACT: This review was prepared to summarize the information on geographic and genetic diversity of *Apismellifera* L. of African honeybees and the progresses made through time and applications of various advanced tools in their taxonomy at subspecies level. African honeybees have developed distinct morphology, behavior and physiology as they evolved in tropical favorable climate. The variations among the African honeybee populations have helped to produce geographic subspecies based on the morphological characteristics. However, the introduction of molecular taxonomic tools has come up with discrepancies in the number of subspecies and lineages, and subspecies composition of evolutionary lineages in Africa and other parts of the world. It has also brought up controversies in the evolutionary history of *A. mellifera* reporting different location as the center of origin of the species at different times: Asia, Africa and Europe. It is not only the tools that are used, but also the genetic variations within subspecies, within colonies in an apiary site and within individuals of the same colony temporally are the factors for continually appearing discrepancies. Natural hybridization between populations of adjacent subspecies along the geographic transition zones and introgression of genes through introduction of colonies that is mainly driven by human demand for desirable traits of honeybees have been among the challenges for discrimination of honeybee subspecies. Therefore, in future genetic diversity and taxonomic investigations, considering these natural variations at subspecies level and developing standardized and integrated procedures from sampling to analysis methods would help to produce generally recognized data base that is critically important to resolve the problem of inconsistencies in taxonomy and nomenclature of honeybee subspecies of Africa and elsewhere.

Key words/phrases: Africa, distribution, diversity, honeybees, population

INTRODUCTION

The honeybees, *Apismellifera* L. are classified into Kingdom, Animalia: Phylum, Arthropoda: Class, Insecta: Order, Hymenoptera: Suborder, Apocrita: Superfamily, Apoidea: Family, Apidae: Subfamily, Apinae (Ruttner, 1988). This order consists of approximately 130,000 recognized species of sawflies, parasitoids, hornets, wasps, ants and bees (Ruttner *et al.*, 1978; Ruttner, 1988). The Family Apidae comprises honeybees, stingless bees, carpenter bees, orchid bees, cuckoo bees, bumblebees, and various other less well known groups (Ruttner *et al.*, 1978), among which only Subfamily Apinae-honeybees and Subfamily Meliponinae-stingless bees produce honey to be worth harvesting (Crane, 1990). *A. mellifera* is commonly known as the European, western or the common honeybee in different parts of the world

and is largely domesticated for honey, pollen, beeswax, propolis, royal jelly and bee venom (Crane, 1990). It is the major agent for global ecology through food production pollination (Allsopp *et al.*, 2008) and has been the main model of scientific social behavioral studies (The Honeybee Genome Sequencing Consortium, 2006).

A. mellifera is distributed in a wide range with various climatic conditions and is subdivided into numerous subspecies (Ruttner, 1988; Engel, 1999). The current understanding of the geographic variation of honeybee is result of accumulation of knowledge over long time (Mexiner *et al.*, 2013), though the existing most comprehensive compilation is the monograph of Ruttner (1988), produced from analysis of numerical taxonomy of traits of classical morphometry. However, since then, numerous investigations using different taxonomic tools and application of several molecular approaches (based on mtDNA, nuclear

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microsatellites, single nucleotide polymorphisms (SNPs)) have been reported with contradictory description of honeybee subspecies and lineages (Garnery *et al.*, 1992; Arias and Sheppard, 1996; Franck *et al.*, 2000; Alburaki *et al.*, 2013). This resulted in describing confusing and continually varying number of honeybee subspecies and subspecies composition of the lineages (Ruttner, 1988; Engel, 1999; Mexiner *et al.*, 2013; Ilyasov *et al.*, 2020). Moreover, the subspecies composition of the evolutionary lineages A (+Z), M, C, O, Y has not yet been resolved (Smith *et al.*, 1991; Garnery *et al.*, 1992; Arias and Sheppard, 1996; Alburaki *et al.*, 2013). Though the picture of Africa honeybees' taxonomy is not very different from that of honeybees elsewhere, this review paper is prepared with intentions of assessing the diversity and geographical distribution of African honeybees. It also discusses the progresses made in characterizing the subspecies of honeybees of Africa and the associated challenges emerged as new technologies of molecular analysis are applied.

Global distribution of honeybees

The natural geographical distribution of the genus *Apis* shows its greatest species diversity in India and adjacent regions, with all of the species except *A. mellifera* that is not found in the region (Ruttner, 1988). Besides, until modern times *Apis* was not found anywhere in the western hemisphere, Australia, or the Pacific except for some of the continental islands such as Japan, Formosa, Philippines, and Indonesia (Roubik, 1989). The earliest classification listed four species of genus *Apis* in the world (Ruttner, 1988; Omer, 2007), which are also economically important ones: the Little honeybee (*Apis florea* Fabricius) – which is native to southeast Asia, the Eastern honeybee (*Apis cerana* F.) – which is native to eastern Asia and is found as far north as Korea and Japan; the Giant honeybee (*Apis dorsata* F.) – which is native to southeast Asia and the Western honeybee (*Apis mellifera* L.) – which is native to Europe, Africa and western Asia (Ruttner, 1988; Roubik, 1989; Crane, 1990). However, the current classification of bees recognizes six more living species that include *A. andreniformis* Smith, *A. koschevnikovi* Enderlein, *A. laboriosa* Smith, *A. nigrocincta* Smith, *A. binghami* and *A. nuluensis* Tingek (Oti, 1990; Engel, 1999; Arias and

Sheppard, 2005) and fossil species belonging to the genus such as *A. vetusta* Engel and *A. armbrusteri* Zeuner (Engel, 1999); *A. henshawi*, *A. cuenoti* and *A. lithohermaea* (Borst, 2015).

A. mellifera is one of the most successful species in the animal kingdom and occupies vast and varied geographical areas. Its native distribution extends from Scandinavia to the Cape of Good Hope and from Dakar in the west to southern Oman in the east and adapts to a very wide range of climatic conditions (Dutton *et al.*, 1981; Ruttner, 1988), covering Europe, West Asia and Africa (Garnery *et al.*, 1992; Arias and Sheppard, 1996). However, there are different and contradicting views of investigators regarding the center of origin of *A. mellifera* to be different continents based on their evidences: Asia (Walberg *et al.*, 2014), Africa (Whitfield *et al.*, 2006) and Europe (Kotthoff *et al.*, 2013). Honeybee colonies are found from sea level to 1000 m in temperate zones; from sea level to 3700 m in the tropics and also reported to survive in the hot and arid zones of Oman at 200 m (Dutton *et al.*, 1981; Ruttner, 1988). Its distribution over vast areas of land with extremely different climates resulted in the diversification of morphological and behavioral characters, which resulted in the evolution of different lineages and subspecies due to long period geographical isolation and ecological adaptation (Hepburn and Radloff, 1998; Arias and Sheppard, 1996). Moreover, honeybees have been under continuous selection and breeding pressure and mass importations of colonies from different areas for high economic performance and desirable behavioral characteristics has led to considerable changes, in the genetic structures of the local populations (Smith, 1991; De la Rua *et al.*, 2009; Meixner *et al.*, 2010).

The diversity and classification of subspecies of *A. mellifera* have been also an issue of continuous ambiguity for many years. Earlier, 24 subspecies of *A. mellifera* were recognized based on morphometric analyses (Ruttner, 1988). According to him, each subspecies is a geographical race characterized with a set of distinctive characteristics that result from local adaptation to the various regions, abiotic factors such as temperature and rainfall and biotic factors such as food, natural enemies and diseases (Hepburn and Radloff, 1998). However, later Engel (1999) reported about 43 subspecies to exist around the world, out of which 10 are found in Africa

(Hepburn and Radloff, 1998; Engel, 1999). Mexiner *et al.* (2013) described *A. m. simensis* as a new and only one subspecies of Ethiopian honeybees arguing all the three previously described subspecies represent only one subspecies of different population, hence making the total number of African subspecies 11. Later, without giving any justification or specific subspecies that had been cancelled or merged, they reported 27 subspecies of honeybees in the world (Mexiner *et al.*, 2013). It seems the number will continue to grow as recent reports have described 31 by Techer *et al.* (2017) and 33 by Ilyasov *et al.* (2020) number of subspecies around the globe.

Origin and diversity of honeybees

It was widely thought that honeybees evolved in the north-eastern Africa and the Near East during the mid-tertiary period of the Pleistocene epoch and rapidly evolved and spread fast to various parts of the world (Ruttner, 1988). This period is known for its Pleistocene glaciation in Europe followed by climatic changes including the creation and expansion of deserts and changes in the floral composition and distribution in Africa that isolated populations and caused diversification and distribution of honeybee lineages and subspecies (Ruttner, 1988; Roubik, 1989).

The honeybees migrated from their center of origin to Africa and Europe in three different evolutionary lineages: A, M and C ("A" colonized south and central Africa, "M" occupied North African and West European, and "C" invaded North Mediterranean) (Ruttner *et al.*, 1978). In response to diversity of habitats, climatic conditions, and flora as well as separation factors, *A. mellifera* has split into numerous subspecies about 0.3-1.3 million years ago (Ruttner 1988; Roubik, 1989). According to the global phylogeography and morphometric analysis of Ruttner *et al.* (1978), the three lineages constitute 24 subspecies. According to these authors, the subspecies of branch A includes the subspecies from Africa (*A. m. lamarckii*, *A. m. jemenitica*, *A. m. litorea*, *A. m. scutellata*, *A. m. monticola*, *A. m. adansonii*, *A. m. unicolor*, *A. m. capensis*), branch M, which includes the subspecies from northern Europe (*A. m. mellifera*), Spain and Portugal (*A. m. iberica*), and northern Africa (*A. m. intermissa*, *A. m. sahariensis*, *A. m. major*), and branch C, which

includes the subspecies from eastern Europe, northern Mediterranean and the Middle East. Later, Ruttner (1988) came up with fourth evolutionary branch, called "O", which was represented by honeybees distributed in the Near and Middle East and divided lineage "C" into two groups, branch C, which included subspecies from Italy (*A. m. ligustica*), Austria and Yugoslavia (*A. m. carnica*), northern Greece and Bulgaria (*A. m. macedonica*), southern Greece (*A. m. cecropia*) and Sicily (*A. m. sicula*), and branch O, which serves as a basal group for the species and included 7 subspecies from the Caucasus mountains (*A. m. caucasica*), Armenia (*A. m. armeniaca*), Iran, Iraq, Syria, and Turkey (*A. m. meda*), Turkey (*A. m. anatoliaca*), Syria (*A. m. syriaca*), Cyprus (*A. m. cypria*) and Crete (*A. m. adami*).

Subsequent molecular characterizations of honeybees such as from mitochondrial DNA also confirmed the distribution of the four lineages from morphologically delineated regions (Garnery *et al.*, 1992, 1993; Arias and Sheppard, 1996; Franck *et al.*, 2000). Moreover, very wide nuclear microsatellite investigations majorly indicated similarity with the distribution of honeybee races from the morphological and mtDNA studies, with few divergences (Estoup *et al.*, 1995; Franck *et al.*, 2000). However, the finding of the lineage "O" through molecular markers created discrepancy in distribution of the subspecies between the branches M and A in the Iberian Peninsula (Smith *et al.*, 1991; Garnery *et al.*, 1995). The subspecies of Northwestern Africa *A. m. intermissa* and *A. m. sahariensis* which were originally assigned to branch M based on morphometrics were reassigned into branch A (Garnery *et al.*, 1992; Arias and Sheppard, 1996). *A. m. lamarckii* from branch A and *A. m. syriaca* from branch C were also reassigned to branch O (Arias and Sheppard, 1996). Therefore, the previous thought that stated honeybees from northern Africa and from Western Europe followed the same evolutionary route and placed them in lineage M was abandoned.

Recently, two more evolutionary branches the "Y" lineage from Ethiopia, represented by *A. m. jemenitica* (Franck *et al.*, 2001) and the "Z" lineage from Syria (Alburaki *et al.*, 2013) have been described. These findings and the successive information changed the hypothesis of center of origin of honeybees stating that *A. mellifera* originated in eastern tropical Africa, spread to northern Europe and east into India and Tien Shan

range in China (Whitefield *et al.*, 2006). However, the evolutionary history of the Western honey bee is far from being resolved. Others who conducted genome sequence comparisons conclude that honey bees originated from Asia and not Africa (Walberg *et al.*, 2014). Yet, a Middle Eastern origin also has its supporters, who based their conclusions on mitochondrial genome analyses (Garnery *et al.*, 1992) and evidence for a North African or Middle East origin was presented by Cridland *et al.* (2017). Even the view that the genus *Apis* originated in Europe and spread from there into Africa and Asia is entertained and supported by fossil specimens discovered in central Europe and Baltic region (Kotthoff *et al.*, 2013). The situation is complex and confusing and no universally acceptable conclusion has yet been reached (Borst, 2015).

Classification of African honeybees

Although the taxonomic description of honeybees from the African continent dates back to 1804 when the French entomologist Latreille named a bee from Senegal *Apis adansonii* (Meixineret *et al.*, 2000), the study of honeybees from this continent remained superficial and incomplete until far into the 20th century. For more than 150 years, *A. m. adansonii* was used as the only name for all the bees of Sub-Saharan Africa (Fletcher, 1978) although morphological variations, along the geographic variability, of the African honeybees were observed starting in 1920's (Ruttner, 1976). The available information about the classification of African honeybees are cumulative of almost a century long collections of reports of regional studies of various investigations. Currently, the African honeybees consist of 10 morphometrically described honeybee subspecies belonging to three lineages A, O, Y (Hepburn and Radloff, 1998; Franck *et al.*, 2000; 2001). These subspecies are geographical races with their own demarcated geographical distributions (Hepburn and Radloff, 1998).

A. m. sahariensis (Baldensperger, 1932) was the first honeybee described in the Maghreb region. These bees have distinguishable yellowish-reddish pigmentation (Ruttner, 1976; 1988). They are distributed immediately to the south of the Atlas Mountains and bordering the Sahara (Ruttner, 1976; Hepburn and Radloff, 1998). On the other hand, there are small and black bees along the

Mediterranean coast of northern Morocco, Tunisia and Northwestern Algeria, named *A. m. intermissa* (Maa, 1953), reputedly aggressive and swarms frequently, rearing over 100 queens in each swarming period (Barour *et al.*, 2011). A single transect running from the Sahara to the Pyrenees by Hepburn and Randloff (1996) also delineated two distinct morpho-clusters corresponding to the *intermissa* and *sahariensis*. *A. m. sahariensis* and *A. m. intermissa* were originally assigned in to lineage M (Ruttner, 1976; 1988), but later in to lineage A, after mtDNA analysis (Garnery *et al.*, 1992, Franck *et al.*, 2001). Moreover, a few populations from hybrid zones between the two races and areas from Spain which form separate intermediate morphoclusters have been reported (Smith *et al.*, 1991; Garnery *et al.*, 1995; Hepburn and Randloff, 1996). Ruttner (1976) earlier also reported that *A. m. major* as a distinct bee race of the Rif Mountains but later he and other authors abandoned it, recognizing it was only an ecotype.

A. m. lamarckii (Cockerell, 1906) was first described in Egypt, but later discovered to be naturally distributed in Egypt and the Sudan along the Nile Valley (El-Banby, 1977). This race is well known for its conspicuous color pattern of bright orange bands with shining black margins on the abdomen and its white pubescence (Eimanifar *et al.*, 2017). Ruttner (1988) reported *lamarckii* belonged to the "A" lineage rather than the North African races primarily because of its yellow pigmentation and small, slender body, which later the mtDNA analysis assigned it into lineage "O" (Arias and Sheppard, 1996). This subspecies is highly defensive and inferior honey producer (Soliman and Sheppard, 2003). Many studies indicate that *A. m. lamarckii* is genetically highly diffused race due to extensive and continuous introductions of temperate honeybee races to Egypt for over a century and intensive cross-breeding to produce commercial bee lines to increase beekeeping productivity (Dewitz *et al.*, 1994).

Since Latreille described honeybees from Senegal as *adansonii* in 1804, honeybees from west and central Africa were described as *A. m. adansonii* (Fletcher, 1978). *A. m. adansonii* are small, yellow bees with aggressive behavior found throughout West Africa extending from Senegal to Namibia, deep into the Sahel of Mali and Burkina Faso as well as into the tropical rain forests of the Congo basin and Zambia (Fletcher, 1978; Ruttner,

1988). This race is generally known for its lack of morphological differentiation though extreme color variations have been observed in Ivory Coast, Guinea, Togo and Benin and as far as to Angola (Hepburn and Radloff, 1998). This race also demonstrates expression of morphological clusters of hybridization all along its border with *A. m. jemenitica* (Radloff *et al.*, 1997).

The honeybee from Cape coast was described by various authors but Eschscholtz designated the name *A. m. capensis* (Eschscholtz, 1821) (Ruttner, 1988). The morphological similarity between *A. m. capensis* and *A. m. scutellata* was a problem for many honeybee taxonomists including Ruttner, to differentiate morphologically (Ruttner, 1976). He then introduced new reproductive physiological characters of *A. m. capensis*: occurrence of workers laying diploid eggs, size of the spermatheca and ovariole count (Ruttner, 1976, 1988). Therefore, the classification of this subspecies is the first to employ the lytokous parthenogenesis and high ovariole numbers in workers (Hepburn and Radloff, 2002). These made it possible to define the geographic distribution and hybridization zone of the two races (Hepburn and Crewe, 1990; Hepburn *et al.*, 1998; Hepburn and Radloff, 1998).

As single and unique honeybee race, *A. m. unicolor* (Latreille, 1804) first described in and endemic to Madagascar also colonizes other small neighboring islands of Seychelles, Mauritius and Reunion (Ruttner, 1988; Hepburn and Radloff, 1998). It is one of the gentlest honeybees in the world and one of the most morphometrically uniform subspecies in Africa (Ruttner, 1988; Franck *et al.*, 2001). Nevertheless, it is reported to be ecologically differentiated into a rather docile type in the highlands and mountains and a more aggressive form along the coastal lowlands (Rasolofoarivao *et al.*, 2015). It is clearly part of the African lineage which mitochondrial haplotype analysis also supported their affinities to other subspecies of Africa (Estoup *et al.*, 1995; Franck *et al.*, 2001). Many authors have suggested that *A. m. unicolor* might have colonized the islands in recent geological times (Estoup *et al.*, 1995; Rasolofoarivao *et al.*, 2015).

A. m. scutellata (Lepeletier, 1836) was first described in South Africa; it is a small bee with a relatively short tongue, it is highly aggressive, swarms and absconds frequently, and is able to nest in a broad range of habitats from cavities to open nests. Though much of their range was

considered to be *adansonii* until Ruttner (1976) proposed that these bees from the savannahs of central and equatorial East Africa and most of South Africa were actually a separate subspecies, *A. m. scutellata*. Therefore, its range starts from Ethiopia, all through the central and East Africa, to the south and southeast of Namibia and Angola, Botswana and Northern Cape of South Africa (Ruttner, 1988; Crewe *et al.*, 1994; Hepburn and Radloff, 1998). This subspecies is also recognized for its highly migratory behavior and in the savannas of East Africa it ascends up to the mountains and hybrids with the black mountain bee *A. m. monticola* and migrates down to the East African coasts to hybrid with the *A. m. litorea* (Smith, 1961; Mexiner *et al.*, 1994; Hepburn and Radloff, 1998).

One of the important races of honeybees in East Africa is *A. m. litorea* first described by Smith (1961) in his transect travel across East Africa countries. He reported the existence of the race in the east coast lowlands of Tanzania and Kenya, though later Ruttner (1976) reported from eastern coasts of Somalia and its distribution extends to the lowland coasts of northern Mozambique (Ruttner, 1988; Hepburn and Radloff, 1998). *A. m. litorea* is small and yellowish bees.

Simultaneously with the discovery of *A. m. litorea*, Smith (1961) described large, dark and docile bees from the cool forests on the slopes of Mt. Kilimanjaro and Mt. Meru between 2400 and 3000 meters which he named *A. m. monticola* (Smith, 1961). Ruttner (1976) also reported the distribution of *A. m. monticola* included the high altitude bees of Mt. Elgon and Mt. Kenya in Kenya as well as, those of the highlands of Ethiopia. Further investigations also confirmed the occurrence of this subspecies in the northern highlands of Ethiopia (Radloff *et al.*, 1997; Amssaluet *et al.*, 2004). Out of East Africa mountains, *monticola* like morphocluster was reported earlier in Cameroun, West Africa too (Ruttner, 1988; Radloff and Hepburn, 1997b).

A. m. jemenitica Ruttner, 1976 is the only subspecies that naturally inhabits two continents, Africa and Asia, was first described from Arabian honeybees (Ruttner, 1976). In both continents, it is distributed in a very vast region extending about 4500 KMs, from western Sahel in western tip of Mali and southeastern Mauritania through the Horn of Africa to the Arabian Peninsula (Al-Ghamdi *et al.*, 2013). Like its ecological

distribution, the morphological variability, biological and behavioral adaptations of this honeybee race are highly diversified. It is so adapted to high temperature variations and erratic and low rainfall (Ruttner, 1988; Al-Ghamdi *et al.*, 2013). The recent discovery of a new evolutionary lineage Y might be related to these wide agroclimatic adaptations of this race or because of its closeness to the Asian populations, which create possibilities for repeated natural gene flow. Literatures from various investigators of African honeybees show the classifications of the subspecies have been inconsistent as different names, including *A. m. nubica* (Ruttner, 1976), *A. m. sudanensis* (El-Sarrag *et al.*, 1992), and *A. m. bandasii* (El-Sarrag *et al.*, 1992; Radloff and Hepburn, 1997a) have been applied to different populations of *A. m. jemenitica*.

The Horn of Africa, physiographically and climatologically a complex region, appears to be very interesting particularly with high honeybee diversity (Whitfield *et al.*, 2006; Meixner *et al.*, 2011). Ruttner (1976) provided the first morphometric account of bees from the hot and arid regions of Sudan and Somalia and named them *nubica*. But subsequent studies reported a significant variation in size, color and behavior among the honeybees of Sudan. For instance, El-Sarrag *et al.* (1992) reported only two subspecies for the region: *sudanensis*, distributed throughout Sudan south of the Nubian Desert and *nubica* along the Sudan-Ethiopia border. The issue of classification of Sudanese honeybees seems unresolved. The recent morphological and mitochondrial analysis evidences argued that the Sudanese bees should be categorized as *A. m. jemenitica*, *A. m. sudanensis* and *A. m. bandasii* (Omer, 2007). Others argue that the mitochondrial analysis of these bees shows a totally new combination and proportion of mitochondrial haplotypes and lacking any presence of lineage "Y" that is represented by *A. m. jemenitica*, thus suggesting the need for new groups to be named separately (El-Niweiri and Moritz, 2008).

Similarly, the Ethiopia honeybee fauna have long been recognized as particular in Africa and has been subjected to several studies leading to controversial interpretations (Mexiner *et al.*, 2011). For instance, Ayalew (1990) reported *A. m. monticola*, *A. m. jemenitica*, *A. m. litorea*, *A. m. adansonii* and *A. m. abyssinica* from different agroclimatic zones. The occurrence of *A. m.*

adansonii, *A. m. litorea* and *A. m. abyssinica* in Ethiopia was reported by this author and never after. Radloff and Hepburn (1997a) also recorded *A. m. jemenitica*, *A. m. bandasii* and *A. m. sudanensis*; however, later considered *A. m. bandasii* and *A. m. sudanensis* only ecotypes of *A. m. jemenitica* (Hepburn and Radloff, 1998). One of the most comprehensive multivariate analyses of Ethiopian honeybees showed the occurrence of new morphocluster named as *A. m. woyigambellato* describe colonies from two localities in southwest part of the country (Amssalu *et al.*, 2004). However, just like that of the Sudan, the controversy of classification of honeybees of Ethiopia seems to be non-ending as more discrepant results continued to be reported. Mexiner *et al.* (2011) suggested that all honeybees from Ethiopia be described as a new and only one subspecies, *A. m. simensis* based on multivariate morphometrical analyses, arguing that the Ethiopian bees are entirely different from subspecies found in the neighboring countries that were also noticed earlier by others (Radloff *et al.*, 1997; Amssalu *et al.*, 2004). Therefore, recognition of this newly described subspecies makes the total number of African subspecies 11 (Mexiner *et al.*, 2011; Techer *et al.*, 2017).

The usefulness of classical morphometric studies and multivariate techniques of analysis remains well established for intraspecific classification of honeybees and described as geographic races, even though environmental effects might modify the expression of the genotypic characters that are polygenic in origin (Ruttner, 1988; Hepburn and Radloff, 1998; Meixner *et al.*, 2011). Because of the limitations of morphometric analysis in the measurement of genetic diversity, molecular techniques based on mtDNA, nuclear microsatellites, single nucleotide polymorphisms (SNPs) have been integrated to honeybee phylogenetic and taxonomic studies (Garnery *et al.*, 1992, 1993; Arias and Sheppard, 1996; Franck *et al.*, 2000). However, the application of these advanced techniques have both supported and contradicted the intraspecific classification of honeybees based on morphological characterization. The honeybee subspecies have been affiliated or discriminated variously or assigned to different evolutionary lineages by different methods (Garnery *et al.*, 1995; Arias and Sheppard, 1996; Franck *et al.*, 2001; Techer *et al.*, 2017). Therefore, it is crucial to develop analytical methods that are integrative of all available tools and standardize the procedures

that may start from defining the geographical zone of a subspecies to sampling and analysis protocols for classification of honeybee subspecies (Meixner *et al.*, 2013). For instance, Radloff and Hepburn (1998) indicated that the greater the distance between samples, the more distinct the morphoclusters are as morphocluster formation may be sensitive to sampling distance. Moreover, application of geometric morphometry that employs software to analyze the geometry of the wing venation landmarks integrated with microsatellite analysis have resulted in purely classifying different races and even detecting hybrids between them (Oleksa and Tofilski, 2014).

Population diversity within subspecies

The adaptations of African honeybees

The honeybee is a highly polytypic species distributed over very wide geographical regions with diversified morphological, behavioral, biological and genetic characters among the subspecies, and even among strains or ecotypes (Hepburn and Radloff, 1998; Meixner *et al.*, 2000; Franck *et al.*, 2000, 2001). The fact that, even though some of these characters are inherited, morphological characters of honeybees are shaped by the environmental factors and exhibit continuous geographical variations were noticed long ago (Hepburn and Radloff, 1998). Moreover, the biological and behavioral variations observed among and within honeybee subspecies are driven by natural selection factors such as temperature, rainfall, availability and distribution of food, prevailing competition and predation (Ruttner, 1988; Roubik, 1989; Hepburn and Radloff, 1998).

There are several major climatic distinctions between Africa and temperate affecting the seasonal cycles of honeybee colonies. Tropical honeybees do not experience severe cold seasons and may forage throughout much of the year (Crane, 1990). Africa has a more propitious climate that is perennially conducive to some flowering, honeybee flight and comb building (Hepburn and Radloff, 1998). The extended foraging season and emphasis on pollen collection may be associated with the high swarming rates and migration of tropical honeybees (Fletcher, 1978; Ande *et al.*, 2008). The phenology of the honeybees seriously follows the seasonal shift of the environmental factors like rainfall and phenology of the major honeybee plants (Roubik, 1989; Crane, 1990) and

among African honeybee, these interconnected phenologies show shifts when colonies start issuing reproductive swarming in response to variations in climatic zones (Hepburn and Radloff, 1998). On the other hand, predation pressure in Africa is severe and must inevitably have selected for greater colony mobility through absconding and migration and too much defensiveness (Roubik, 1989).

Tropical honeybees, especially the African evolved ones have been characterized by distinct morphology, behavior and physiology compared to temperate honeybees (Ruttner, 1988; Roubik, 1989). It is known that African honeybees are smaller, lighter in color, more aggressive, with higher tendency to reproductive swarming, migration, absconding, pollen and propolis collection (Fletcher, 1978; Hepburn and Radloff, 1998; Hunt, 2007; Ande *et al.*, 2008). For instance, African bees are reported to have greater resistance to the pathogens and parasites (Dietemann *et al.*, 2009), though the genetic basis of this phenotypic variation is largely unknown.

Population diversity within subspecies of African honeybees

African honeybees exhibit the morphological, behavioral and physiological variations among the subspecies in association with local adaptations in response to various environmental factors (Smith, 1961; Fletcher, 1978, Ruttner, 1988, Hepburn and Radloff, 1998). However, these variations are not only limited to the subspecies level but also observed within ecotypes, colonies in an apiary site and temporally and simultaneously intracolony (Hepburn and Radloff, 1996; Garnery *et al.*, 1992; Radloff *et al.*, 2003; Amssalu *et al.*, 2004).

Morphological variations in altitude and latitude

Ruttner (1988) showed that a long-distance honeybee geocline of morphological characters and geographic latitude extends from North to South along the countries in Scandinavia to the Cape tip of South Africa. It was shown that latitude significantly affects body pigmentations and size, hair length and density, length of tongue and hind legs (Hepburn and Radloff, 1998). Similar correlations exist between morphological characters and altitude, indicating the presence of short-distance ecoclines (Smith, 1961; Ruttner,

1988; Meixner *et al.*, 1994). Body size and color are also highly correlated with altitude, as continuous and gradual decrease is observed in body size and color change from dark to lighter as we descend altitude; thus, the highland honeybees are generalized to be larger and darker than the lowland ones (Ruttner, 1988; Radloff and Hepburn, 1997a; Amssalu *et al.*, 2004). Some investigators tried to justify the morphological and genetics difference in honeybees of the highlands of Ethiopia and Kenya from the surrounding lowland savannah is related to the altitude and associated factors (Hepburn and Radloff, 1998; Meixner *et al.*, 2000).

Biological variations

Migration

The adaptation of seasonal migratory behavior by tropical honeybees is considered as survival strategy (Roubik, 1989). The seasonal migration of Sub-Saharan Africa honeybees in dry months was reported for *A. m. jemenitica* (Nuru *et al.*, 2002); *A. m. litorea* (Smith, 1961); *A. m. capensis* (Hepburn and Radloff, 1998); *A. m. adansonii* (Fletcher, 1978); *A. m. scutellata* (Nuru *et al.*, 2002; Amssalu *et al.*, 2004); *A. m. monticola* (Meixner *et al.*, 1994; 2000). Lack of food and water, overheating and fire were reported to be the major causes of migration of tropical Africa (Fletcher, 1978; Hepburn and Radloff, 1998). Different races and ecotypes show varied levels of tendency toward migration and also respond differently to migration inducing factors. For instance, *A. m. jemenitica* was reported to be highly migratory in the Sahel region and in Ethiopia, while no migration was observed in north Oman and Yemen (Dutton *et al.*, 1981; Hepburn and Radloff, 1998; Nuru *et al.*, 2002).

Reproductive swarming

Honeybees swarm as part of the colony's reproductive process (Roubik, 1989). The extended foraging season and emphasis on pollen collection may be associated with the high swarming rates of tropical honeybees (Fletcher, 1978; Ande *et al.*, 2008). The difference between honeybee subspecies and agroclimatic conditions also determine the performance of colonies in terms of swarm initiation (Ruttner, 1988; Crane, 1990). Geographical regions of *A. m. scutellata* and *A. m. jemenitica* were identified to be of high swarming incidence in Ethiopia (Nuru *et al.*, 2002). In the

contrary, even some highly populous colonies in the same apiary site are reluctant to develop swarm cells (Nuru and Dereje, 1999). Moreover, colonies of the same races of *A. m. scutellata*, *A. m. adansonii*, and *A. m. capensis* showed variations in time shift in reproductive swarm as the result of adaptations to local ecological and climate conditions, in addition to varied number of queens produced (Fletcher, 1978; Hepburn and Radloff, 1998).

Temperament

The African honeybee is very aggressive though some tend to sting less than others and are more docile (Fletcher, 1978; Roubik, 1989; Hunt, 2007). They respond more rapidly and intensely than European bees and usually sting in larger numbers (Hunt, 2007). However, it has been argued that with frequent handling they become used to being inspected and therefore are mildly aggressive (Hunt, 2007). The fact that differences in the level of defensiveness observed among different groups of African honeybees are due to the genetic variations has been recognized (Fletcher, 1978; Hepburn and Radloff, 1998). For instance, genetically and behaviorally separate groups of bees have high variations in alarm pheromone levels (Collins *et al.*, 1989). These variations in defensiveness were also observed to the level of inter and intracolony levels (Fletcher, 1978). Moreover, climatic factors mainly temperature, is one of the prominent environmental factors to determine the level at which bees react to the provoking stimulus (Fletcher, 1978). As a result, honeybees are believed to be more docile at cool, high altitude areas than hot lowland areas (Corner, 1985).

Genetic variations

Intracolony and intercolony variations

It was Alpatov in 1930's who first recognized not only the occurrence of inter colony and intracolony variations in honeybees, but also the average variability of morphological traits observed intracolony is less than that of intercolony variations in an apiary (Ruttner, 1988). There is an innate difference in character variation that shows some traits are more conserved while others continually change (Radloff *et al.*, 2003), as the nature and the degree of variability in phenotypic traits are supported by

their genetic inheritance and environmental influence (Cornuet and Garnery, 1991).

The phenomenon called polyandry, which is the repeated mating of the queen with several drones from different colonies, is one important factor to cause the intracolony and intercolony level variations (Page, 2013). This is due to the fact that a honeybee colony is a set of members (casts) of family with full and half-sisters from different drone lines (Oldroyd *et al.*, 1992). Individuals with mixed color pattern in a colony and colonies with different proportions of predominantly black or yellow pigmentation in the same location were observed in Ethiopia (Hepburn and Radloff, 1998; Amssalu *et al.*, 2004). It is common to see the color of the workers of even a colony is mixed sometimes and changes over a period of time. This is mainly due to the different traits acquired from multiple mating of queens with drones of differing allelic frequencies (Oldroyd *et al.*, 1992; Radloff *et al.*, 2003). Likewise, there are significant variations in the ratios of the constituents in the bouquets of sting gland pheromones intercolony (Garnery *et al.*, 1992) and intracolony (Hepburn and Radloff, 1996) for honeybees of North Africa. There are also seasonal variations in temperament of a colony associated with the availability of resources in the beehive and the seasonal cycle of the colonies: season of brood rearing and honey storage that aggravate more aggressiveness (Hepburn and Radloff, 1998).

Inter population genetic variations

Because a population is a breeding group in the genetic sense, the genetic constitution of a population is an array of gene frequencies (Hepburn and Radloff, 1998). And the gene frequencies of a population of honeybees change between generations, due to population size, differences in fertility and viability of progeny, migration and mating systems (Radloff and Hepburn, 2000; Hepburn *et al.*, 2004).

The analysis of mtDNA variance in the COI-COII intergenic region has been recently used to separate among the *A. mellifera* races and to infer phylogenetic relationships (Cornuet and Garnery, 1991; Smith, 1991; Garnery *et al.*, 1992, 1995; Estoup *et al.*, 1995; Franck *et al.*, 2000, 2001). Most of African honeybees are categorized under a mitochondrial lineage "A" (Arias and Sheppard, 1996; Franck *et al.*, 2001) though there is

considerable mtDNA variability, particularly for honeybees of the northeast of the continent. Significant variations are observed between and within the evolutionary lineages geographically from the very small variations in local populations to the level of recognizable and separate subspecies (Cornuet and Garnery, 1991; Franck *et al.*, 2000, 2001). For instance, *A. m. intermissa* were assigned into the A lineage constituting eight different haplotypes (A1–A4, A8–A10, A13), while the close race *A. m. sahariensis* constitutes five haplotypes (A1, A3, A4, A8, A9) of the same lineage (Garnery *et al.*, 1995). The honeybees of Northeast Africa are assigned to three different mtDNA lineages: A, O and Y. In Egypt *A. m. lamarckii* is associated only with the lineage O, while *A. m. jemenitica* of Ethiopia has been categorized under lineage Y; and *A. m. litorea* of Somalia are categorized under lineages O and A, respectively (Franck *et al.*, 2001). Such splitting of the previously recognized Y lineage into A and O has also been recently reported in the Sudan (El-Niweiri and Moritz, 2008). These authors argued that honeybees of the dry regions were genetically more similar to *A. m. lamarckii* that belongs to lineage O and the populations from the wet regions were more alike *A. m. adansonii* belonging to lineage A.

On the other hand, the ecologically isolated subspecies from tropical and southern Africa (*A. m. adansonii*, *A. m. monticola*, *A. m. scutellata*, *A. m. capensis* and *A. m. unicolor*) could be only slightly discriminated by applying analyses of molecular allozymes (Meixner *et al.*, 1994). The significant mtDNA difference among these races observed came from only the level of the proportion of haplotypes A1 and A4 (Franck *et al.*, 2001). This low level molecular differentiation among African races could be justified by high tendency to seasonal migration, absconding and reproductive swarming of these races (Meixner *et al.*, 1994, 2000). The studies by Rasolofoarivao *et al.* (2015), Techer *et al.* (2017) showed that the honeybees of Madagascar and adjacent islands are almost 100% comprised of the A1 haplotype, making it very legitimate to infer that the possibility of migration is reduced in islands.

A High degree of polymorphism of nuclear markers is common in the African honeybees (Estoup *et al.*, 1995; Franck *et al.*, 2001; Eimanifar *et al.*, 2018). Genetic diversity indicators like allelic richness, the average heterozygosity and average

number of alleles, microsatellite loci genetic diversity are significantly higher in African honeybee subspecies (Estoup *et al.*, 1995; Franck *et al.*, 2001; Kibogo, 2017; Eimanifar *et al.*, 2018). Factors associated with high levels of nuclear polymorphism include quaternary climate changes that drove diversification and expansion of races on the continent (Franck *et al.*, 2001), potential of building up big and effective population that supports higher alleles in the population (Estoup *et al.*, 1995), and the high mobility character of colonies (Hepburn and Radloff, 1998; Meixner *et al.*, 1994, 2000). Many investigators argue that this is one of the strategic advantages for African honeybees as colonies that are genetically diverse tend to form strong colonies faster by swarming, are more resistant to pests and diseases, and are better suited to their environment and this has an effect on improved hive productivity (Mattila and Seeley, 2007).

Hybridizations and introgression

In addition to the adaptive strategies of African honeybees that favor high mobility and fast population growth (Fletcher, 1978; Hepburn and Radloff, 1998), its physiography does little to form strict natural barriers to prevent continuous gene flow among adjacent honeybee populations, except the Saharan desert that created vast land free of bees and Atlas Mountains (Roubik, 1989; Hepburn and Radloff, 1998). As a result, hybridization zones between adjacent populations characterized by gradient changes in morphometric and molecular features are very common. Transition zones have been observed between *A. m. adansonii* and *A. m. jemenitica* of Africa (Radloff and Hepburn, 1997b), *A. m. sahariensis* and *A. m. intermissa* (Hepburn and Radloff, 1996), *A. m. capensis* and *A. m. unicolor* and *A. m. capensis* and *A. m. scutellata* (Hepburn and Radloff, 1998, Hepburn *et al.*, 1998). In eastern and Central Africa, hybridization between *scutellata* and *monticola* (Meixner *et al.*, 1994); and *A. m. scutellata*, *A. m. litorea* and *A. m. monticola* horizontally across savanna areas and vertically between savanna areas and the mountain bees was reported (Meixner *et al.*, 2000; Kibogo, 2017).

Among the African honeybees, however, it is not only hybridization between adjacent races that is continuously changing the population genetic structure of the local honeybees. Introgression of genes through introduction of colonies that is

mainly driven by the human demand for desirable traits of honeybees has also been observed in African honeybees since long time (Hepburn and Radloff, 1998; El-Niweiri and Moritz, 2008). Such introduced honeybee genes from European colonies have been detected among *A. m. lamarckii* colonies in Egypt, among Libyan bees, Sudanese honeybees and *A. m. scutellata* colonies in South Africa (Dewitz *et al.*, 1994; El-Niweiri and Moritz, 2008; Shaibi *et al.*, 2009; Meixner *et al.*, 2010) and the two South African subspecies (Hepburn *et al.*, 1994).

CONCLUSIONS

The honeybees are such beneficial insects to human existence in terms of pollination ecosystem services, honey and beeswax production. *A. mellifera* L. is a cosmopolitan and extremely diversified species with wide distribution in Africa, Europe and Asia. Recently, this species is categorized into six evolutionary lineages; A, M, C, O, Y and Z with their own geographical distributions. The African honeybees belong to the lineages A, O and Y represented by 10 or 11 subspecies that are clearly discriminated morphoclusters distributed in delineated geographical representations that are mainly the result of local ecological adaptations to the climatic factors that are driving population isolation and genetic diversity, though there are overlaps due to hybridization and mobility. The molecular techniques like mtDNA and other nuclear markers recently applied in the systematics of African honeybees also confirm the occurrence of these separate biogeographical races. However, the complex region of Horn of Africa might have exceptionally high genetic mixing up from the inland and also continuous gene flow from the Arabian Peninsula, shows instability in description and taxonomy of honeybee populations of the area and witnessed repeated rejections of described honeybee races by various investigators. In this region there are issues that are yet not settled. The morphometric description of *A. m. simensis* needs conformation of molecular analysis. Moreover, the subspecies composition of the evolutionary lineages A (+Z) and O reported for *A. m. jemenitica* from the Sudan and *A. m. litorea* from Somalia should be resolved through comprehensive molecular evidences.

The general evolutionary adaptation strategies of high mobility and fast and high population growth by African honeybees have been important factors in resulting continuous genetic intermixing and increased diversity, even though hybridization zones characterized by both continuous phenotypic and genotypic expressions make classifications of the African honeybees at the subspecies level difficult. In fact, most of the classification methods do not work universally and what works best in one region fails to discriminate subspecies. Therefore, this makes the use of combination of all available techniques significantly important. However, the population genetic of the honeybees is so diversified that the behavioral, biological and morphological characteristics of population show tremendous variations not only among the subspecies, but also among different agro-ecologies, among colonies of the same location and even intracolony within the subspecies. These high genetic diversities have their own merits in resisting diseases and pests and maintain the survival potential of colonies and hence need detail investigations to understand the genetic basis of these merits and conserve them for better utilization.

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