

PHYLOGENETIC ANALYSIS OF THE BEE TRIBE ANTHIDIINI

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ABSTRACT

The phylogenetic relationships among members of long tongue bee tribe Anthidiini (Megachilidae: Megachilinae) were investigated at the Department of Entomology and Wildlife, University of Cape Coast (Ghana) and the Agricultural Research Council, Pretoria (South Africa) from July, 2006 to May, 2007. Ten museums located in three continents loaned the 990 specimens used for the studies. Thirty-three ingroup taxa out of the 37 known genera of the world's Anthidiini and two outgroup genera, were included in a cladistic analysis of the tribe based on 51 adult external morphological characters. The cladograms yielded three major clades which have been erected as subtribes for the world's Anthidiini, namely Afranthidina, Anthidina and Euaspina. Monophyly of the Anthidiini was confirmed. Based on the result of the cladistic analysis and biogeographic data, it is suggested that the origin and center of radiation for the Anthidiini probably occurred in the southern pantropic region (Southern Africa to South East Asia) of Gondwana. A second and independent migration to the Neotropical region by the probable ancestors of the current Neotropical genera is also conceivable.

INTRODUCTION

Among the hymenopteran insects, bees occupy a unique position in the world of Arthropods. They are entwined into most aspects of human culture and mythology, not to mention our agriculture, economy and general ecology.

The Anthidiini is one of four bee tribes of the subfamily Megachilinae and family Megachilidae. The others are: Megachilini, Dioxyini and Osmiini. This is an enormous subfamily of bees with thousands of species that are commonly called leaf-cutter, carder and mason bees. The anthidine bees exhibit considerable

variation in form from heriadiform, hopliform, chalicodomiform, megachiliform to anthophoriform. Members of this tribe mostly have yellow integumental markings (O'Toole and Raw, 1991; Michener and Griswold, 1994; Michener, 2000). The tribe consist of thirty seven genera (see appendix 1b), each with between one and twelve subgenera.

Anthidine bees are widely distributed, occurring on all continents, except Antarctica, and fairly common, except with only one species known from Australia. The tribe largely comprises pollen collecting bees; the few parasitic

groups (*Afrostelis*, *Euaspsis*, *Hoplostelis* and *Larinostelis*) have narrow ranges of host taxa within the families Apidae (tribe Euglossini) and Megachilidae (genus *Megachile*, Latreille (Michener and Griswold, 1994, Michener, 2000)).

In the Western Palaearctic Region and South Africa, the anthidines have been recorded as principal pollinators of certain plant families including the Labiatae, the Compositae, the Leguminosae and the Zygophyllaceae (Armbuster and Steiner, 1992; Müller, 1996). In Ghana, members of this bee tribe are known to be the principal pollinator of plants belonging to the mint family (Labiatae) and probably many other food crops and wild plants. However, data on the phylogeny of all the anthidine genera of the world is fragmentary and very limited to few geographic regions. It is therefore important to study the phylogenetic relationship among members of this tribe, in order to ascertain the monophyly of the tribe and probably postulate a better classification system for the tribe.

MATERIALS AND METHODS

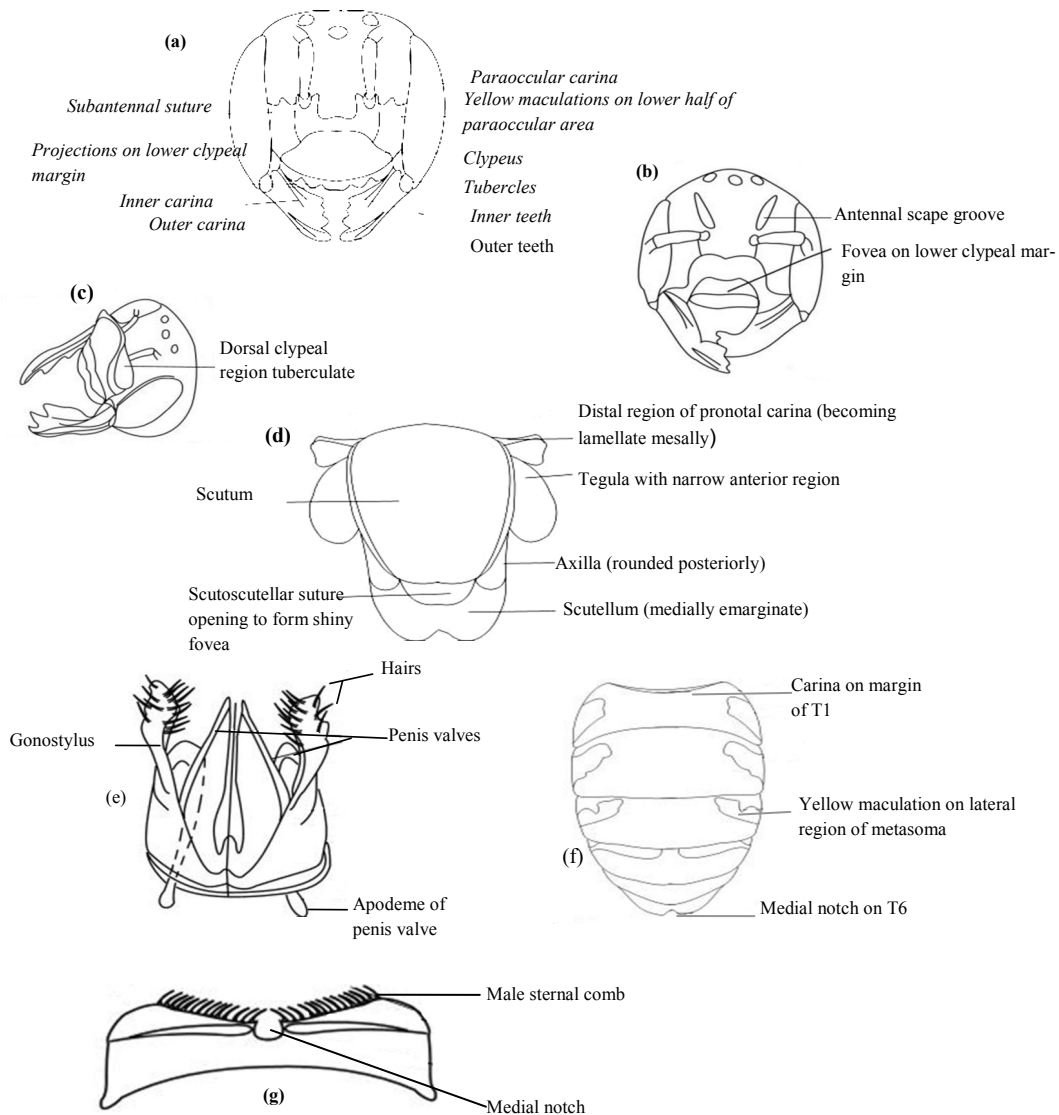
A total of 990 bee specimens belonging to 35 genera were obtained from ten museums across the world from July, 2006 to May, 2007. The genera and the total number of specimens examined (in parenthesis) are as follows: *Lithurgus* (2), *Megachile* (2), *Acedanthidium* (2), *Afrantheidium* (111), *Afrostelis* (29), *Anthidiellum* (162), *Anthidioma* (2), *Anthidium* (63), *Anthidioctes* (2), *Apiantheidium* (1), *Aspidosmia* (2), *Aztecantidium* (2), *Bathantidium* (1), *Benanthis* (1), *Cyphantidium* (3), *Diantheidium* (16), *Duckeantidium* (2), *Eoantidium* (37), *Epantheidium* (4), *Euaspsis* (40), *Gnathantidium* (3), *Hoplostelis* (2), *Hypantidiodes* (2), *Hypantidium* (6), *Icterantheidium* (2), *Indantidium* (2), *Neantheidium* (2), *Notantidium* (2), *Pachyantidium* (49), *Parantidium* (2), *Plesiantidium* (245), *Pseudoantidium* (140), *Rhodantidium* (7), *Serapista* (32), *Trachusa* (13).

Immediately the loaned specimens were re-

ceived from the various museums, they were quarantined and curated. Quarantine procedures involved storage of specimens in freezers at temperatures of -4°C to -15°C for a minimum period of two weeks to eliminate possible museum pests such as mites. In certain instances, direct hand picking method was used to remove these pests. Curating of specimens was carried out to ensure that the detailed information on each specimen conformed to what was provided by literature, as well as ensuring that each specimen was accurately placed into the appropriate taxon. The morphological examination of the specimens in this present study was on visual observation of external morphological features. Specimens were examined, and illustrations and measurements made using an Olympus dissection microscope fitted with a drawing tube and a graticule. Male genitalia and hidden sterna of some relaxed specimens were removed using a pair of forceps and macerated in 10% KOH for 12–24 hours at room temperature, depending on the thickness of the cuticle of each structure. After clearing the genitalia and sterna were stored and examined in 75% glycerine. The terminology used in this study follows that of Michener (1944, 2000) and Michener and Griswold (1994). Different morphological terms are explained in the text.

The study incorporated 35 species representing 33 anthidine genera (ingroup, listed in the matrix, see appendix 1b) and two outgroups namely *Lithurgus pullatus* (Vachal) and *Megachile semiflava* (Cockerell). Roig-Alsina and Michener (1993) postulated that, these outgroup used represent the recent common ancestor and the most recent common ancestor of the tribe Anthidiini. Hence, these two outgroups revealed the probable transformation series of the character states within the subfamily Megachilinae.

Adults of each species were examined based on 51 external characters, and each character for which distinct states occurred in different species was included in the matrix (see appendix 1b). A number of the characters used in the



Figures 1a-g. Labelled integumental structures, showing the diagnostic characters used for the analyses. (a) Dorsal view of face of genus *Anthidiellum*. (b) Face of genus *Notanthidium*, indicating the antennal scape groove behind antennal socket and fovea on the ventral region of clypeus of the female (Dorsal view). (c) Lateral view, face of female *Aspidosmia*, showing the protuberance of the dorsal region of clypeus. (d) Mesosoma of *Anthidiellum* (*Pygnanthidiellum*), in dorsal view. (e) Male genitalia of *Pleisanthidium*. (f) Dorsal view, metasoma of *Anthidiellum orichalscopatum*. (g) S5 of male *Anthidiellum otavicum*, showing the male sternal comb.

present phylogenetic analysis of the tribe were taken or modified from Roig-Alsina and Michener (1993). Annotated lists of the characters used in the phylogenetic reconstructions can be found in the Appendix 1a. The states of each character within the ingroup were coded as 0 representing primitive; 1, 2 and 3 referring to successive derived states. All character states in the outgroup were considered to be plesiomorphic. Additive coding was employed for the various character states. Unknown character states were coded with "?" whereas inapplicable characters were coded with "-". All characters were weighted equally. Four characters of the male genitalia were deactivated during the analysis.

Character matrix for cladistic analyses were constructed using the WinClada computer software, version 1.00.08 (Nixon, 1999-2002). The phylogeny was created in NONA computer software (Goloboff, 1993) using an unconstrained heuristic search strategy having Multiple TBR+TBR (mult* max*). The search parameters were 100,000 maximum trees to keep (Default=100), 500 replication (Default=1), 500 starting tree per replication (Default=1) and zero (0) random time. Common cladogram measures such as the consistency index (CI) and the retention index (RI) were used to evaluate the fitness of the data to the cladogram. Analysis of characters as well as character optimization using unambiguous optimization modus was performed with the WinClada software.

Bootstrap analysis (Felsenstein, 1985) and jackknife sampling (Lanyon, 1985; Siddall, 1996) were used to assess evidential support for clades of cladograms. Values were calculated based on 100 replicates with 50 random sequence additions per replicate. Presentation of trees as well as character mapping was carried out in the WinClada.

RESULTS

The results obtained from the cladistic analysis of the data matrix (see Appendix 1b) using the heuristic search tool yielded 191 equally most

parsimonious trees with a length (L) of 86 steps, the consistency index (CI) of 56 and the retention index (RI) of 83. Figure 2 shows a typical tree topology of most of the 191 most parsimonious trees obtained during analysis of characters and character states by NONA computer software. However, the strict consensus tree in figure 3 yielded L: 93 steps, CI: 52% and RI: 80%. On the strict consensus tree 17 unsupported nodes were collapsed into several polytomies. In addition, bootstrap and jackknife search strategies resulted in maximum branch length, consistency and retention indices of 90/89 steps, 54/55% and 81/81%, respectively (Figures 4 and 5).

Three major clades parallel to the outgroups (*Lithurgus* and *Megachile*) emerged from the cladograms (Figures 4-5) as follows: (1) *Aspidosmia*, *Afranthidium*, *Anthidioma* and *Neanthidium*. (2) *Afrostelis*, *Hoplostelis*, *Anthidiocetes*, *Hypanthioides*, *Cyphanthidium*, *Paranthidium*, *Eoanthidium*, *Epanthidium*, *Euaspis*, *Serapista*, *Apianthidium*, *Aztecantidium* and *Duckeantidium*. (3) *Anthidium*, *Dianthidium*, *Icteranthis*, *Anthidiellum*, *Bathanthidium*, *Gnathanthidium*, *Pachyanthidium*, *Pseudoanthidium*, *Acedanthidium*, *Benanthis*, *Hypanthidium*, *Indanthidium*, *Notanthidium*, *Pleisanthidium*, *Rhodanthidium* and *Trachusa*.

During the statistical analysis of the data matrix by NONA (computer software), approximately 308 maximum steps and 53 minimum steps of character change occurred. However, 47 out of the 51 external morphological characters were actually employed (activated), while the remaining four characters on male genitalia were deactivated. In addition, 4 characters of male genitalia of species *Anthidioma murinum* resulted as inapplicable characters "-" due to the fact that, this genus is known by only female species. 18 character states on males from five genera were revealed as missing characters. The five genera included *Icteranthis*, *Bathanthidium*, *Gnathanthidium*, *Aztecantidium* and *Duckeantidium*. The 18 characters states were examined on the genitalia of the male specimens of these genera. However, only

type specimens of those genera were available for examination hence, examinations were not conducted on hidden structures such as the male genitalia that require dissection procedures. In genera *Bathanthidium* and *Gnathanthidium* only female specimens were available. During the preliminary analysis of the tribe by NONA, the absence of these characters was observed to have caused the collapse of several nodes into polytomies. Hence, all those characters were deactivated during the analysis of the tribe in the course of the main research analysis by NONA.

The acronyms attached to each taxa in all cladograms give an indication of the biogeographic data of that genus. Thus, AF represents the Afrotropical Region (Sub-Saharan, East and West Africa and Madagascar); PA refers to the Palearctic Region (North Africa and Europe); OR represents the Oriental Region (Asian countries); NE represents the Neotropical Regions (South America) and COS refers to the Cosmopolitan regions (those genera that occur in all the geographical areas).

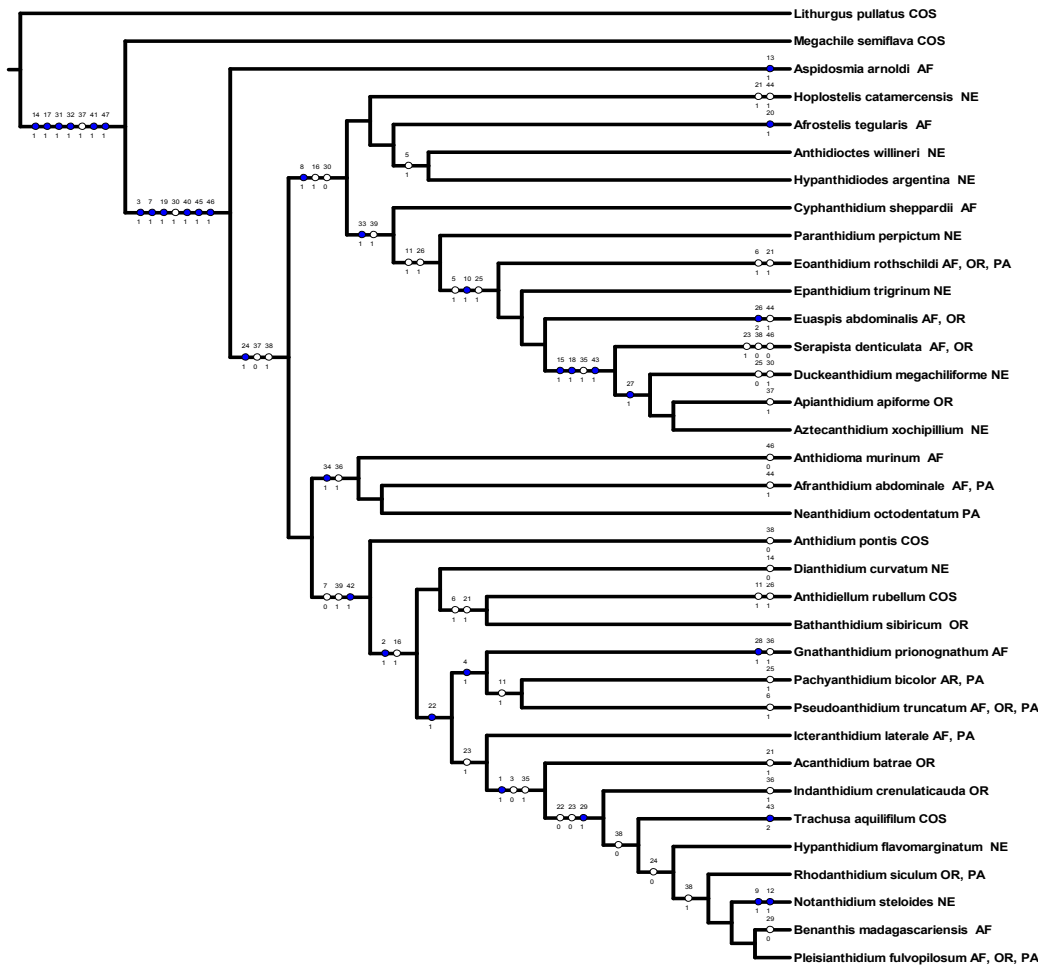


Fig. 2: Cladogram showing the hypothetical phylogeny of the tribe using the heuristic search tool

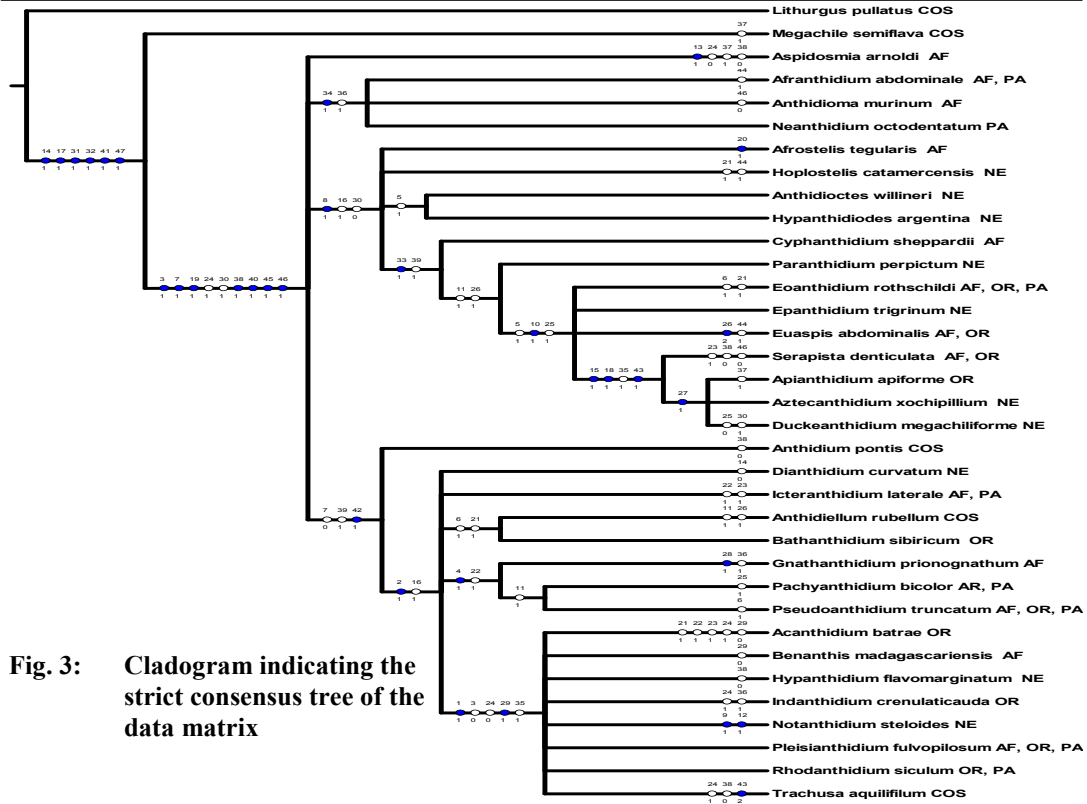


Fig. 3: Cladogram indicating the strict consensus tree of the data matrix

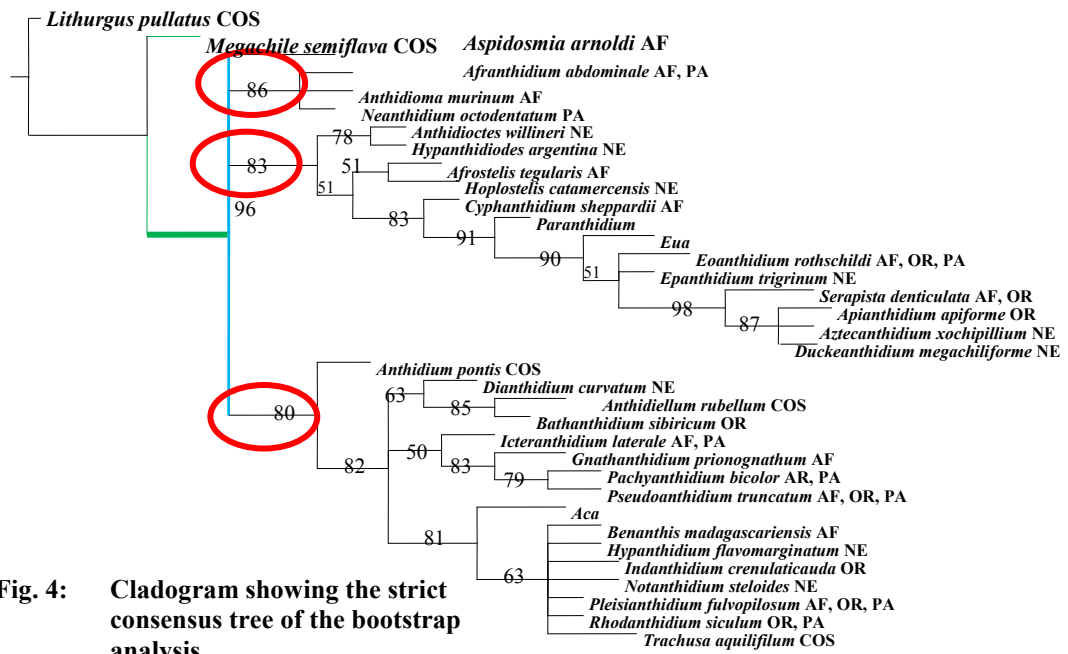


Fig. 4: Cladogram showing the strict consensus tree of the bootstrap analysis

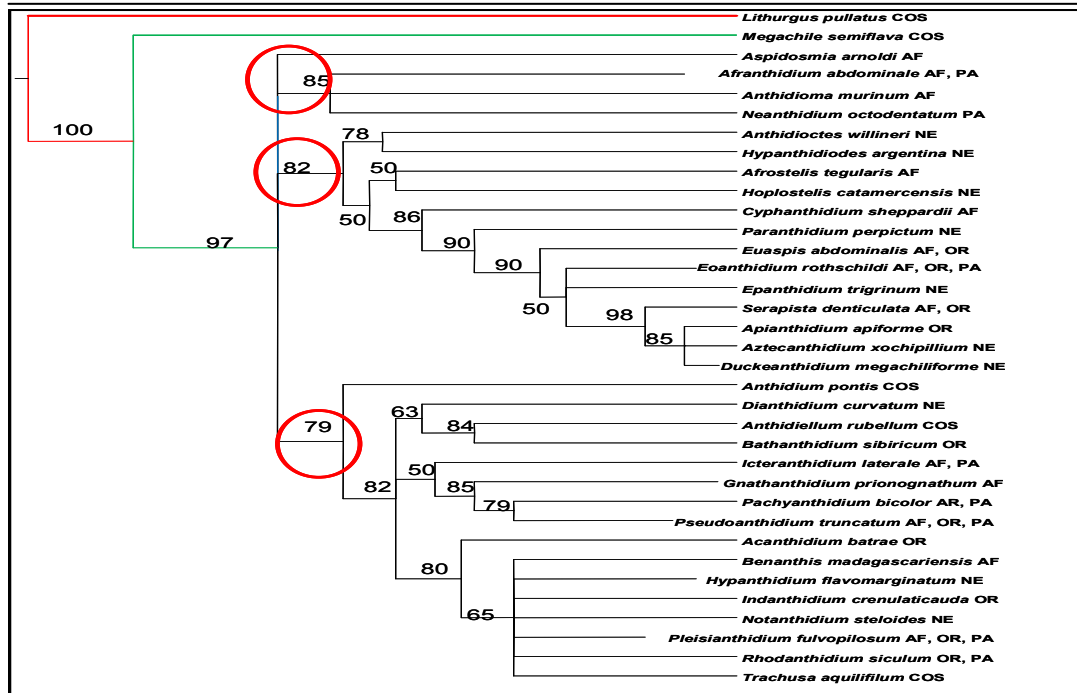


Fig. 5: Cladogram showing the strict consensus tree of the jackknife analysis of clades

DISCUSSION

Monophyly of tribe and its genera

The tribe Lithurgini is reported as the sister group of the Osmiini, Anthidiini, Megachiliini and Dioxyini which constitute the Megachilinae, with the tribe Megachilini known to the closest sister group of the Anthidiini (Riog-Alsina and Michener, 1993). These authors adequately demonstrated the Megachilinae to be monophyletic by the presence of several unique synapomorphies. However, they did not demonstrate the monophyly of the Anthidiini using most of the genera of the tribe. The inclusion of *Lithurgus pullatus* (Vachal) and *Megachile semiflava* (Cockerell) in this study as two outgroups clearly confirmed the monophyly of the Anthidiini. This is supported by nine synapomorphies, seven of which are non-homoplasious characters. Two characters however showed reversals in higher tree topology and therefore probably do not strictly characterize the Anthidiini as a whole. The Anthidiini is clearly supported by several unambiguous syn-

apomorphies and by very high bootstrap and jackknife values, hence confirming the tribe as a monophyletic taxon.

The first major island comprises relatively small genera, namely *Aspidosmia*, *Afranthidium*, *Anthidioma*, *Neanthidium*, with very high bootstrap and jackknife values of 86% and 85% in the strict consensus trees. *Aspidosmia* forms the first major clade within this island. Its monophyly is demonstrated by an autoapomorphy, that is, the dorsal region of the female clypeus being highly elevated. This uniquely derived autoapomorphy is supported by another character state namely, second recurrent vein of the forewing entering the second submarginal cell. The clade therefore supports the relationship of genus *Aspidosmia* to the rest of the anthidine bees which confirms previous classification works including Peter (1972), Riog-Alsina and Michener (1993), Michener and Griswold (1994) and Michener (2000).

The second major clade comprises three para-

sitic genera and nine non-parasitic. The parasitic genera include *Afrostelis*, *Euaspis* and *Hoplostelis* while the non-parasitic genera comprise *Anthidioctes*, *Hypanthioides*, *Cyphanthidium*, *Paranthidium*, *Eoanthidium*, *Epanthidium*, *Serapista*, *Duckeanthidium*, *Apianthidium* and *Aztecanthidium*. This island is clearly supported by high bootstrap and jackknife values of 83% and 82% respectively and gives an indication that these genera form a close sister group.

The remaining major island of the Anthidiini comprised *Anthidium*, *Dianthidium*, *Anthidiellum*, *Bathanthidium*, *Icteranthis*, *Gnathanthidium*, *Pachyanthidium*, *Pseudoanthidium*, *Acedanthidium*, *Benanthis*, *Hypanthidium*, *Indanthidium*, *Notanthidium*, *Pleisanthidium*, *Rhodanthidium* and *Trachusa*. This island is supported by high bootstrap and jackknives values (80/ 79 %) and is also defined by a combination of two characters. The collapse of several nodes within this clade could probably be due to inadequate characters to clearly resolve the clade.

In spite of a number of missing genera and character states during the analysis of the tribe, at least the following classification, based on available information, is suggested for the tribe. Most of the generic classification of Michener (2000) is retained, except that, three main subtribes are erected within the tribe. The genus *Hypanthioides* is to be synonymized with *Anthidioctes* (the latter being the older available name). In addition, synonymization of the genus *Bathanthidium* with genus *Anthidiellum* was considered, however, they were retained as separate taxa. The tribe Anthidiini may therefore be placed under family Megachilidae and subfamily Megachilinae with three subtribes, namely Afranthidina, Anthidina and Euaspina. The subtribe Afranthidina comprises non-parasitic, small to medium size, megachiliform to anthophoriform and hairy bees which are all endemic to the African continent. The four genera that occur in this subtribe include *Afranthidium*, *Anthidioma*, *Aspidosmia* and *Neanthidium*. The Anthidina comprises mainly non-parasitic bees with wide morphological varia-

tion. Its size ranges from medium to large and varies in shape from hoplitiform, megachiliform, chalicodomiform to anthophoriform. It is the only subtribe with three cosmopolitan genera (*Anthidium*, *Anthidiellum* and *Trachusa*) while the other genera (*Acedanthidium*, *Bathanthidium*, *Benanthis*, *Dianthidium*, *Gnathanthidium*, *Hypanthidium*, *Icteranthis*, *Indanthidium*, *Notanthidium*, *Pachyanthidium*, *Pleisanthidium* and *Pseudoanthidium*) are known to have narrow range of distribution. The subtribe Euaspina constitute both parasitic and non-parasitic genera, just as in Anthidina, it varies from small to large size hoplitiform to anthophoriform bees, as well as small heriadi-form bees. The subtribe is made up of genera with limited geographic distribution among the continents. These genera include, *Anthidioctes*, *Afrostelis*, *Apianthidium*, *Aztecanthidium*, *Cyphanthidium*, *Duckeanthidium*, *Eoanthidium*, *Epanthidium*, *Euaspis*, *Hoplostelis* and *Serapista*. The classification of the Anthidiini into three subtribes enormously facilitates its study and provides useful information on these genera. The name of each subtribe was derived from the oldest available name within the genera.

Biogeography and Evolution of Anthidiini

Three main lineages of Anthidiini are distinguished. The first lineage comprises a relatively small clade of mostly Afrotropical genera (*Afranthidium*, *Anthidioma* and *Aspidosmia*), and a Palaeartic genus *Neanthidium* (Algeria and Morocco).

Second lineage is made up of three cosmopolitan genera (*Anthidium*, *Anthidiellum* and *Trachusa*), three genera endemic Neotropic and Nearctic regions (*Dianthidium*, *Hypanthidium* and *Notanthidium*), ten genera occurring in Afrotropical and Palaeartic regions (*Icteranthis*, *Pachyanthidium*, *Gnathanthidium*, *Benanthis*; Palaeartic and Orient: *Rhodanthidium*, *Bathanthidium*, *Acedanthidium*, *Indanthidium*, *Pleisanthidium*

The third clade also comprises five genera endemic to Neotropic and Nearctic regions (*Anthidioctes*, *Hoplostelis*, *Paranthidium*, *Az-*

tecanthidium and *Duckeanthidium*), two Afrotropical genera (*Afrostelis* and *Cyphanthidium*), one Oriental genus, *Apianthidium*, three genera with relatively extensive distribution within the old world (*Euaspis*, *Eoanthidium* and *Serapistia*).

The Afrotropical and Oriental regions exhibit high diversity Anthidiini at the generic level, with 16 and 13 genera, respectively. One subtribe is limited to the African continent. High diversity in these geographic areas at the generic level and basal groups of primitive forms are enough evidence to postulate that, the origin and center of radiation for the Anthidiini probably occurred in southern Pantropic Region (Southern Africa to South East Asia). These geographic locations, before the split of the world's continent, formed South-eastern block of the southern hemisphere, super-continent Gondwanaland (during the early Cretaceous over 150 million years before present, Raven and Axelrod, 1974).

The discovery of the oldest fossil bee, *Melittosphex burmensis* (Melittosphexidae (Pionar and Danforth, 2006)), from the early Cretaceous Burmese amber (~100 million years ago) which is 35-45 million years older than the previously known fossil bee, *Cretotrigona prisca* by Michener and Grimaldi (1988 a & b) and Grimaldi and Engel (2005), tend to suggest that the evolution of bees might have occurred earlier than previously indicated, possibly, as early as during the evolution and diversification of angiosperms in early Cretaceous era. *M. burmensis* shares several characters with most members of present day anthidine genera. Visual observation clearly shows that the forewing of this bee possess strong first and second transverse cubital veins that forms two complete submarginal cells. Further to this, this fossil resin-collecting bee has a heriadiform shape which is typical of small size anthidine bees. Hence, it is probable to also assume that the present day anthidine genera existed about the same era as *M. burmensis*.

It is also known that, it was only in the tertiary (66.4 to 1.6 million years ago) during the late

Paleocene and early Eocene era that bilaterally symmetrical organization of floral organs as in present day, coevolved with animal behaviour including bee sociality (Danforth, 2004; Dilcher, 2006). Based on the discovery of the new fossil bee (after publication of Engel and Perkovsky, 2006), and data from host-plant specialization in western anthidine bees by Müller (1996), as well as information of the evolution and biogeography of bees, it is probable to assume that the basal lineages of Anthidiini happened much earlier, possibly arose during the early Cretaceous, a period when the continental connection of Africa and South-eastern Asia still existed. Further, it is however possible that, more diversification within the tribe took place much later (Eocene-Oligocene period), especially of genus *Anthidium*, accounting for some of the fossils recorded in Engel and Perkovsky (2006).

A second and independent migration to the Neotropical region by the probable ancestors of current Neotropic genera is conceivable. Such an event probably took place when the gap between the drifted continents of South America and Africa was narrow enough to permit short insect flight along its corridors and similar climatic conditions still existed that provided similar forage resources for these bees. Evolution of parasitism within the tribe Anthidiini probably followed this scenario. The parasitic genera probably evolved ones within the lineages of the Anthidiini. This lineage comprises mainly two Afrotropical and Oriental genera (*Afrostelis* and *Euaspis*), and a Neotropic genus *Hoplostelis*.

CONCLUSIONS

Of great interest to this current research are the possible contributions of suggested reclassification and phylogeny of the tribe Anthidiini. This study confirmed the monophyly of the Anthidiini and most of its genera as valid taxa. Further to this, three subtribes: Afranthidina, Anthidina and Euaspina have been erected for the world's anthidine genera. The Afrotropical and Oriental regions exhibit

high bee richness within the Anthidiini at the generic level, with 16 and 13 genera, respectively. One subtribe, Afranthidina is limited to the African continent. High bee richness at the generic level and basal groups of primitive forms are enough evidence to postulate that, the origin and center of radiation for the Anthidiini probably occurred in southern Pantropic Region (Southern Africa to South East Asia).

A second and independent migration to the Neotropical region by the probable ancestors of current Neotropic genera of the Anthidiini is conceivable. In addition, it is suggested that the parasitic genera probably evolved ones within the lineages of the Anthidiini.

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APPENDIX 1a

Characters used for the Cladistic Analyses of the Anthidiini

The following characters and character states were used in the cladistic analysis of Anthidiini (Fig 1a-g)).

1. Paraocular carina. (0) Present. (1) Absent.
2. Dorsal region of preoccipital area, in female. (0) Gently rounded or angulate. (1) Carinate.
3. Lateral region of preoccipital area, in female. (0) Gently rounded. (1) Carinate.
4. Carinate.
5. Preoccipital area. (0) Without lamella. (1) Lamellate.
6. Juxtantennal carina. (0) Absent. (1) Present.
7. Subantennal suture. (0) More or less straight. (1) Curved.
8. Distance between antennal sockets. (0) More than two antennal socket diameters. (1) Subequal to or less than two antennal socket diameters.
9. Antennal scape, posterior surface. (0) Densely pubescent. (1) Sparsely pubescent to impubescent
10. Antennal scape groove of female. (0) Absent. (1) Present.
11. Mediolongitudinal region of face. (0) Slightly raised. (1) Strongly raised.
12. Ventral margin of clypeus. (0) Without projections, smooth. (1) Unevenly margined, with projections.

13. Ventro-lateral region of female clypeus. (0) Flat. (1) Tuberculate.
14. Dorsal region of clypeus of female. (0) Flat to gently convex. (1) Highly elevated.
15. Mandibular teeth of female. (0) Three teeth. (1) Four or more teeth.
16. Malar area. (0) With one lower tubercle. (1) Possess several tubercles.
17. Facial vestiture. (0) Densely pubescence. (1) Sparse to impubescence.
18. Dorso-lateral angle of pronotum. (0) Well developed. (1) Absent or weakly developed.
19. Distal region of pronotal lobe. (0) Round or gently curved. (1) Sharply pointed.
20. Omaulus. (0) Rounded. (1) Carinate.
21. Scutum length. (0) As long as broad. (1) Elongated, twice as long as broad.
22. Scuto-scutellar suture. (0) Similar to scuto-axilla suture, closed. (1) More open than scuto-axilla suture, foveate.
23. Posterior margin of axilla, dorsal view. (0) Does not extend laterally beyond lateral margin of scutum. (1) Extend beyond lateral margin of scutum.
24. Posterior region of axilla. (0) Without spine. (1) Possess spine.
25. Scutellum, dorsal view. (0) Not overhanging and concealing propodeum. (1) Obscures propodeum, at least medially.
26. Scutellum, lateral view. (0). Rounded posteriorly, without lamella. (1) Lamellate posteriorly.
27. Scutellum, posterior margin, dorsal view: (0) Entire. (1) Slightly notched postero-medially. (2) Deeply notched postero-medially.
28. Apex of outer hind tibia spur. (0) More or less straight. (1) Strongly curved.
29. Shape of female hind basitarsus. (0) Slender, subequal to or slightly more than half width of hind tibia. (1) Broader than hind tibia.
30. Length of hind basitarsus. (0) Short, subequal to or less than half length of hind tibia. (1) Long, subequal length of hind tibia.
31. Vestiture on dorsal surface of hind basitarsus. (0) Sparse to impubescent. (1) Dense.
32. Basitibial plate in male. (0) well defined. (1) Very small or absent.
33. Tarsal claws of female. (0) Simple. (1) Cleft, inner ramus sometimes a tooth.
34. Apex of marginal cell position. (0) Separated from wing margin. (1) On wing margin.
35. Apex of marginal cell, shape. (0) Rounded. (1) Pointed.
36. Stigma. (0) Broad, twice as wide as prestigma. (1) Slender, as wide as prestigma or absent.
37. First recurrent vein of fore wing. (0) Extends distally behind first transverse cubital vein. (1) Meets first transverse cubital vein distally or enters first submarginal cell.
38. Second recurrent vein of fore wing. (0) Meets second transverse cubital vein distally or extend distally behind second transverse cubital vein. (1) Enters second submarginal cell midway.
39. Second recurrent vein, in profile. (0) Enters second submarginal cell or meet second transverse cubital vein distally. (1) Extend distally behind second transverse cubital vein.
40. Second cubital cell of fore wing. (0) Subequal half length of first cubital cell or less. (1) More than half or subequal length of first cubital cell.
41. Jugal lobe. (0) Long, more than half length of vannal lobe. (1) Short, half as long as or less than half length of vannal lobe.
42. Apex of jugal lobe: (0) Broadly rounded; (1) Narrowly rounded to slightly pointed.
43. Vein Cu-v of hind wing. (0) Straight. (1) Slants.
44. Vein Cu-v of hind wing (length). (0) Short, much less than half as long as second abscissa of vein M+Cu. (1) Long, subequal to half length of second abscissa of vein M+Cu. (2) Longer than second abscissa of M+Cu.
45. Scopa. (0) Present. (1) Absent.
46. Fovea on anterior region of T1. (0) Present. (1) Absent.
47. Hairy subapical bands on postgraduli on T1-T5. (0) Present. (1) Absent.

48. Pygidial plate on female T6. (0) Present. (1) Absent.
 49. Sternal setae of male. (0) Absent. (1) Present.
 50. Gonostylus. (0) Narrow less than half width of gonocoxite. (1) Expanded more than half width of gonocoxite.
 51. Penis valves. (0) Narrowly separated. (1) Widely separated.
 52. Apical region of penis valves. (0) Without notch on margin. (1) Notched

APPENDIX 1b

Table 1: Data matrix of morphological character state of the tribe Anthidiini indicating taxa, statistics, character and character state of each species

TAXA	CHARACTERS AND CHARACTER STATES				
NTAX= 35	NCHAR=51				
INFORMATIVE: 255	7+9283++06 +001360310 442768205+ 0082+42691 0+42130+++				
MAXSTEPS 308:	8++394++17 +112471421 55387+316+ 1193+537+2 1+632411++				
MINSTEPS 53:	1111111111 1111111111 1111111111 1111111111 1111111111				
WTS 51/51:	1111111111 1111111111 1111111111 1111111111 1111111111				
ACTIVATION:]4: [47:	yyyyyyyyyy yyyyyyyyyy yyyyyyyyyy yyyyyyyyyy yyyyyynnnn				
ADDITIVE -0: +51:	+++++++ ++++++++ ++++++++ ++++++++ ++++++++				
<i>Lithurgus pullatus</i>	0000000000	0000000000	0000000000	0000000000	0000000000
<i>Megachile semiflava</i>	1000000000	0001001000	0000000000	1100001000	10000011010
<i>Acanthidium batrae</i>	0100000000	0001011010	1111000001	1100100111	11001110100
<i>Afranthidium abdominale</i>	1010001000	0001001010	0001000001	1101010101	10011110100
<i>Afrostelis tegularis</i>	1010001100	0001011011	0001000000	1100000101	10001110000
<i>Anthidiellum rubellum</i>	1110010000	1001011010	1001010001	1100000111	11001110000
<i>Anthidiocetes willneri</i>	1010101100	0001011010	0001000000	1100000101	10001110000
<i>Anthidioma murinum</i>	1010001000	0001001010	0001000001	1101010101	1000101-----
<i>Anthidium pontis</i>	1010000000	0001001010	0001000001	1100000011	11001110100
<i>Apianthidium apiforme</i>	1010101101	1001111110	0001111000	1110101111	10101110110
<i>Aspidosmia arnoldi</i>	1010001000	0011001010	0000000001	1100001001	10001110110
<i>Aztecantidium xochapillum</i>	1010101101	1001111110	0001111000	1110100111	10101110???
<i>Bathanthidium sibiricum</i>	1110010000	0001011010	1001000001	1100000111	1100111????
<i>Benanthis madagascariensis</i>	0100000000	0001011010	0000000001	1100100111	11001111110
<i>Cyphanthidium sheppardii</i>	1010001100	0001011010	0001000000	1110000111	10001110010
<i>Dianthidium curvatum</i>	1110000000	0000011010	0001000001	1100000111	11001111000
<i>Duckeanthidium megachiliforme</i>	1010101101	1001111110	0001011001	1110100111	10101110???
<i>Eoanthidium rothschildi</i>	1010111101	1001011010	1001110000	1110000111	10001110000
<i>Epanthidium trigrinum</i>	1010101101	1001011010	0001110000	1110000111	10001111000
<i>Euasps abdominalis</i>	1010101101	1001011010	0001120000	1110000111	10011110100
<i>Gnathanthidium prionognathum</i>	1111000000	0001011010	0101000101	1100010111	1100111????
<i>Hoplostelis catamercensis</i>	1010001100	0001011010	1001000000	1100000101	10011111010
<i>Hypanthidiodes</i>	1010101100	0001011010	0001000000	1100000101	10001111000
<i>Hypanthidium flavomarginatum</i>	0100000000	0001011010	0000000011	1100100011	11001110111
<i>Icteranthidium laterale</i>	1110000000	0001011010	0111000001	1100000111	11001110???
<i>Indanthidium cremulaticauda</i>	0100000000	0001011010	0001000011	1100110111	11001110010
<i>Neanthidium octodentatum</i>	1010001000	0001001010	0001000001	1101010101	10001111000
<i>Noanthidium steloides</i>	0100000010	0101011010	0000000011	1100100111	11001110010
<i>Pachyanthidium bicolor</i>	1111000000	1001011010	0101100001	1100000111	11001111000
<i>Paranthidium perpictum</i>	1010001100	1001011010	0001010000	1110000111	10001111110
<i>Pleisanthidium fulvopilosum</i>	0100000000	0001011010	0000000011	1100100111	11001111100
<i>Pseudoanthidium truncatum</i>	1111010000	1001011010	0101000001	1100000111	11001111000
<i>Rhodanthidium siculum</i>	0100000000	0001011010	0000000011	1100100111	11001111000
<i>Serapista denticulata</i>	1010101101	1001111110	0011110000	1110100011	10101010111
<i>Trachusa aguilifilum</i>	0100000000	0001011010	0001000011	1100100011	11201111100