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Revised classification and phylogeny of an Afrotropical species group based on molecular and morphological data, with the description of a new genus (Coleoptera: Scarabaeidae: Onthophagini)

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Abstract

The worldwide distributed *Onthophagus* genus comprises at present more than 2,000 species, that often show a complicated and uncertain systematic history. In particular, the many Afrotropical species included in this genus have never been entirely reviewed after the division into 32 species-groups proposed by d'Orbigny in 1913, although subsequent research focussing on some of these species suggested that *Onthophagus* constituted a not monophyletic taxon. In order to highlight their phylogenetic relationships, the various Afrotropical species-groups of d'Orbigny must thus be examined, and it would be advisable to study them separately to avoid misunderstanding. In this framework, the taxonomic position of the three species currently included in the 21st d'Orbigny group was examined. Both morphological and biomolecular analyses contributed in confirming that these species (i.e., *Onthophagus caffrarius* d'Orbigny, 1902, *O. quadraticeps* Harold, 1867 and *O. signatus* Fåhraeus, 1857) constituted a well-defined monophyletic group that cannot be maintained within the genus *Onthophagus*. Therefore, the *Kurtops* **gen.n.** is here described to accommodate these Afrotropical species, that are nevertheless always included within the Onthophagini tribe. On the basis of the phylogenetic relationships here elucidated, it was also emphasized that the new genus is strictly related to *Digitonthophagus* and *Phalops*, thus it was proposed to include the three genera into a single clade of suprageneric rank naming it as *Phalops* complex.

Keywords. *Onthophagus*; new genus; *Phalops* complex; molecular analysis; morphological analysis; phylogeny; geometric morphometrics

Introduction

The widespread genus *Onthophagus* Latreille, 1802 comprises more than 2,000 species and is thus one of the largest genera in the world (Emlen et al. 2005). It was hypothesized that these dung beetles originated in Africa during the Oligocene (23-33 MYA) concurrently with the expansion of grassland habitats and the radiation of mammals (Ahrens et al. 2014). They quickly spread from Africa, and now can be found in all continents, with species living in a wide range of exceedingly different habitats and feeding on every kind of dung (Emlen et al. 2005). Such a high biological diversification corresponds to an extreme systematic complexity, that is exemplified by the troublesome taxonomic history not only of the *Onthophagus* genus, but also of the whole Onthophagini tribe.

The more than 700 Afrotropical *Onthophagus* species currently known are still divided (for the most part) into the 32 species-groups proposed by d'Orbigny (1913), who developed a system of dichotomous keys entirely based on characters of external morphology for species recognition. The monophyly of the *Onthophagus* species-groups was not expressly supported by the d'Orbigny compendium, and some of these groups had to be removed from *Onthophagus,* and must be regarded as new entities whose taxonomic rank requires a careful evaluation.

Over the years, a number of new taxa were described in order to accommodate some of those species previously included in *Onthophagus*. A good example is the case of *Digitonthophagus* Balthasar, 1959 that was described (together with others) as a subgenus of *Onthophagus* (Balthasar 1959, 1963) and later elevated to generic rank (Zunino 1981). Yet again in recent years more controversial classifications within the Afrotropical *Onthophagus* d'Orbigny groups was developed (Moretto 2009; Tagliaferri et al. 2012), but a lot remains unresolved due to the well-known species richness and complexity of this megadiverse genus. As a result, not only the *Onthophagus* genus, but the entire d'Orbigny classification system of Afrotropical Onthophagini is now under scrutiny.

Within this framework, we focused on the 21st group, that includes only three small species, recorded from the Southern Africa subregion: *Onthophagus caffrarius* d'Orbigny, 1902, *O. quadraticeps* Harold, 1867 and *O. signatus* Fåhraeus, 1857. The species-group was defined by a set of characters related to external morphology, that are not exclusive to this group (d'Orbigny 1913), as the base of pygidium with a transversal carina, or the pronotum covered by granules or granulate points which can both be found in the majority of *Onthophagus* groups (d'Orbigny 1913).

The question about the ambiguous taxonomic position of the 21st group has been recently raised in the context of studies dealing with the review of phylogenetic relationships within Scarabaeinae by the use of a biomolecular approach. In their phylogenetic review of the Madagascar dung beetles Wirta *et al.* (2008) placed *O. signatus* (a species of 21st d'Orbigny group) very close to *Phalops wittei* (Harold, 1867) and *Digitonthophagus gazella* (Fabricius, 1787), all these species being however well-separated by both Oniticellini and other Onthophagini. The latter was thus regarded as not monophyletic, with at least two distinct clades recognized within this tribe. In addition, Monaghan *et al.* (2007) and, more recently, Mlambo *et al.* (2015) showed that the clade *Digitonthophagus* and *Phalops* Erichson, 1848 are sister to all the other Onthophagini, although neither of them included the species of the 21st d'Orbigny group in the analysis. Based on this research, it was hypothesized that *Phalops* and *Digitonthophagus* constitute a separate clade from the other Onthophagini previously examined, and were closely related. However, the taxonomic position of the 21st species-group of *Onthophagus* was not verified in those studies.

The uncertain taxonomic position of *Phalops* and *Digitonthophagus* within Onthophagini was also highlighted by studies in which various morphological characters were analyzed and discussed. The male genitalia (formed by the aedeagus and endophallus) have been recently examined in various Onthophagini groups (Tarasov and Solodovnikov 2011; Medina et al. 2013; Tarasov and Génier 2015), giving remarkable results especially in defining the endophallus sclerites, although the homologies of *Digitonthophagus* and *Phalops* were not fully defined (see the online Supplementary Material for further details). Other internal morphological structures that have not been employed till now (for instance the female genitalia and the epipharynx) could bear phylogenetic signals, and surely deserve a careful examination, to determine their usefulness to solve major taxonomic and phylogenetic problems within the Onthophagini.

The aim of the present paper was to evaluate the taxonomic position of the species of the *Onthophagus* 21st group within Onthophagini and verify the suggested hypothesis of its close relationships to *Phalops* and *Digitonthophagus*, according to former findings. The present research employed both molecular (COI sequences) and morphological (external and internal anatomical traits) approaches, focusing also on the recognition of novel structures useful in the assessment of the phylogenetic relationships among these taxa.

Material and Methods

A diversified approach was chosen to evaluate the hypothesis that the species included in the *Onthophagus* 21st group constituted a monophyletic and separate taxon, more closely related to *Phalops* and *Digitonthophagus* than to the other *Onthophagus* taxa. The results obtained from the different methods (i.e., biomolecular taxonomic distance analysis, morphological phylogeny and geometric morphometrics analysis) were then compared.

A dataset was established that included *Phalops*, *Digitonthophagus*, *Onthophagus* 21st species-group, and some other representatives of *Onthophagus* from Afrotropical and Palearctic regions. The Oriental species *Serrophorus seniculus* (Fabricius, 1781), belonging to the *Proagoderus* complex (Tarasov and Kabakov 2010) was chosen as the outgroup taxon in the phylogenetic analyses.

In detail, the following species were examined: *Digitonthophagus bonasus* (Fabricius, 1775); *D. gazella* (Fabricius, 1787); *Euonthophagus flavimargo* (d'Orbigny, 1902); *Onthophagus caffrarius* d'Orbigny, 1902; *O. quadraticeps* Harold, 1867; *O. signatus* Fåhraeus, 1857; *O. nigriventris* d'Orbigny, 1902; *O. (Onthophagus) illyricus* (Scopoli, 1763); *O. (Palaeonthophagus) coenobita* (Herbst, 1783); *O. (Palaeonthophagus) medius* (Kugelann, 1792); *O. (Palaeonthophagus) nuchicornis* (Linnaeus, 1758); *O. (Palaeonthophagus) ovatus* (Linnaeus, 1767); *O. interstitialis* (Fåhraeus, 1857); *O. bituberculatus* (Olivier, 1789); *O. depressus* Harold, 1871; *Phalops ardea* (Klug, 1855); *P. boschas* (Klug, 1855); *P. prasinus* (Erichson, 1843); *P. rufosignatus* van Lansberge, 1885; *P. wittei* (Harold, 1867).

Molecular analysis

The molecular analysis focused on mitochondrial cytochrome oxidase I (COI), a powerful tool for characterizing taxa (Hebert et al. 2003, 2004; King et al. 2008; Dincă et al. 2013) commonly employed for species identification at a molecular level, and the core of an integrated taxonomic system (i.e., the DNA barcoding, see Casiraghi et al. 2010; Dincă et al. 2015; Vodă et al. 2015). COI sequences of various Onthophagini species collected from GenBank were employed to provide a dataset comprising 21 sequences from 14 species (see Table 1 for the list of species employed in the analysis, their acronyms and accession codes).

Multiple sequence alignment was performed using the MUSCLE method as implemented in MEGA v6 (Tamura et al. 2013), then the alignment of sequences was checked manually. All positions containing gaps and missing data were eliminated during the subsequent analyses, that were made using MEGA v6, except when otherwise stated.

To test the genetic divergence among these taxa, a distance matrix was calculated employing the Kimura 2 parameter (K2P) correction, claimed as the best DNA substitution model for low genetic distances (Nei and Kumar 2000; Casiraghi et al. 2010), and commonly used to evaluate the barcode gap among taxa. Standard error estimates were obtained by the bootstrap procedure (Nreps = 1,000). The threshold value between intra and interspecific distances (i.e., the barcode gap) was established at 1%, which is commonly used as the level of separation in most previous studies of animals (Ratnasingham and Hebert 2007, 2013; Chevasco et al. 2014; Del Latte et al. 2015).

Phylogenetic reconstruction *via* Nearest-Neighbor-Interchange (NNI) was applied to generate an automatically-computed NJ tree using the Tamura-Nei (TN93) parameter substitution model (Nei and Kumar 2000) with all positions containing gaps and missing data eliminated from the dataset (complete deletion option). This initial tree was set as default for phylogenetic reconstruction *via* the Maximum Likelihood (ML) method coupled with bootstrapping reliability tests (Nreps = 1,000). Support for internodes was assessed by bootstrap percentages.

The branch supports were evaluated by both approximate likelihood ratio test (SH-like aLRT) and non-parametric bootstrap (Nreps = 1,000) methods (Simmons 2014), as implemented in PhyML 3.1 (Guindon and Gascuel 2003; Guindon et al. 2010), applying the same settings of the former ML analysis (single initial BioNJ tree; TN93nucleotide substitution model; no discrete gamma model; equilibrium frequencies optimised; NNI tree topology search).

To test the monophyly of clades, the MUSCLE-aligned matrix was analyzed by phylogenetic networks analysis (PNA) as implemented in SplitsTree 4.14.2 (Huson and Bryant 2006). Constant (N = 166), gapped (N = 286) and non-parsimony informative (N = 336) sites were excluded from the analysis. Monophyly of the lineages was assessed by the Neighbor-Net (splitstransform = EqualAngle) method (Bryant and Moulton 2004), whereas bootstrapping estimates (1,000 runs) were employed to support the splits.

Morphological analysis

More than 1,500 specimens were examined to determine morphological characters that support inter and intraspecific differences among the Onthophagini taxa, with a special focus on the *Onthophagus* group 21 species and related groups.

The material examined was loaned from the following Museum collections:

MHNL – Musée des Confluences, Lyon, France
NMEG – Naturkundesmuseum, Erfurt, Germany
MNHN – Muséum National d'Histoire Naturelle, Paris, France

We also examined material from private collections of E. Barbero (EBCT - Torino, Italy), and P. Moretto (PMCT - Toulon, France).

Various external and internal morphological traits were carefully examined, according to the suggestions of the most recent literature (Tarasov and Solodovnikov 2011; Tarasov and Génier 2015) that emphasized the necessity to find novel morphological characters to elucidate phylogenetic relationships within the Scarabaeoidea.

The mouthparts and genitalia of both sexes were dissected and treated following the methods usually employed to prepare slides (Barbero *et al.* 2003). The images of the internal and external structures were then captured using a Leica® DMC4500 digital camera connected to a stereoscopic dissecting scope (Leica® Z16Apo).

The nomenclature of the anatomical traits adopted in this study follows those used in Tarasov and Solodovnikov (2011), Tarasov and Génier (2015) and Roggero *et al.* (2015).

The datasets obtained by observation of the various structures have been employed to carry out two different analyses, a morphological phylogeny and a geometric morphometric analysis.

Among the various structures examined, some were selected to build the matrix for the subsequent phylogenetic analysis (see the characters list below), although others were discarded. In particular, the antenna was not used in the present analysis since it proved to be very complicated structurally and difficult to interpret. Although the cavity identified by Tarasov and Solodovnikov (2011) can be easily detected on the 12th and 13th antennal segments (Fig. 1A-C) of the species studied here, it is apparently extremely variable and can appear as either a more or less concave or convex area. The shape of this area is not constant even in the same species (Fig. 1D-E). Although the antennal cavity is an extremely interesting structure, its functions have to be studied further in detail.

Male genitalia are currently employed in the systematics of Onthophagini, but their features remain to be fully elucidated. They are constituted by an aedeagus and an inflatable endophallus which extends into the female bursa copulatrix during copulation (House and Simmons 2003). On the inside membrane of the endophallus there are various sclerites, that were recently examined and named by Tarasov and Solodovnikov 2011 (see online Supplementary Material for further details).

Unlike the male genitalia, widely employed in insect systematics for many years, the female genitalia are much less studied, despite the hypothesized co-evolution among these structures. As pointed out in evolutionary biology studies, male and female genitalia are subject to a stabilizing selection to enforce mate recognition and reproductive isolation at a specific level (Eberhard 1992; Gilligan and Wenzel 2008; Mikkola 2008; Masly 2012; Wojcieszek and Simmons 2013). As female genitalia must co-evolve in concert with those of males to allow coupling, phylogenetic signals of genitalia must follow the same trend in both sexes (Simmons and Garcia-Gonzales 2011). The female genitalia in Onthophagini are structurally relatively simple. They consist of a membranous sac-like vagina, carrying a more or less sclerotized support area (the infundibular wall, variously shaped), and a receptaculum seminis for the storage of sperm, connected to the vagina by the infundibular tube (House and Simmons 2005; Pizzo et al. 2006, 2008).

The epipharynx constitutes the upper part of the mouth, with the function of food filtration. It is an extremely complex structure formed by a membranous part and a sclerotized part with a support role. Due to extreme diversification of features, the epipharynx has proved a very useful tool to generate separation of groups at different taxonomic levels, giving often highly meaningful results as regards phylogenetic signals (Barbero et al. 2003; Roggero et al. 2015).

Phylogenetic analysis

The selected structures (i.e., head, pronotum, elytra, legs, mentum, epipharynx, and genitalia of both sexes) were employed to build a matrix of 35 binary and multistate characters (Table 2, and see the online Supplementary Material for a detailed discussion of the endophallus sclerites).

The character list can be found in the Supplementary Material.

The matrix of 35 morphological characters (set as unordered and equally weighted) was analyzed by Maximum Parsimony Analysis (heuristic search) in PAUP 4.0b.10 (Swofford 2002) using the software default settings (stepwise addition with simple addition sequence, tree bisection – reconnection branch swapping, ACCTRAN character-state optimization). The multistate characters were interpreted as “uncertainty”, and the gaps treated as “missing”. The MaxTrees limit was set to automatically increase from the initial setting. Trees were rooted by the outgroup method, and the strict consensus was calculated. After the first run, the characters were reweighted by the rescaled consistency index (successive weighting) and heuristic searches were performed until the character weights no longer changed and trees with identical length were found in three consecutive searches (stability in the trees). The

Newick output trees obtained in the former analysis were visualized with FigTree v1.4.2 (Rambaut 2014).

Statistical support for each branch was assessed by PAUP using the non-parametric bootstrap method (Felsenstein 1985), with the same heuristic search settings as above, and 100,000 replications.

The morphological dataset was also analyzed using TNT (Goloboff et al. 2003, 2008). Both Implicit Enumeration and Traditional Search options were employed using the default settings with the Implied Weighting set to ON. The synapomorphies common to all trees were mapped onto the resulting trees. Tree statistics were calculated using a TNT script (stats.run). Relative support values were calculated within TNT by symmetric resampling, bootstrap standard and jackknife with 1,000 iterations (Sharkey et al. 2012).

The Bayesian inference of phylogeny (Markov chain Monte Carlo simulations, or MCMC) was used to approximate the posterior probabilities of trees and parameters, as implemented in MrBayes v3.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2011). The analysis was initiated with a random starting tree and run for 2,500,000 generations (two runs, eight chains), sampling trees every 100 generations, with rate heterogeneity modelled by an equal distribution. Posterior clade probabilities were used to assess nodal support. The trees sampled during the burn-in phase (i.e. before the chain had reached its apparent target distribution) were discarded (25% of the total). The remaining trees were summarized in the Bayesian Majority Rule consensus trees, and the topologies of the two runs were compared to detect differences.

For the graphic exploration of MCMC convergence in Bayesian phylogeny, TRACER v1.6 (Rambaut et al. 2013) was then employed to analyze the results obtained from Bayesian MCMC runs. Trends that might suggest problems with MCMC convergence were checked and the lnL probability plot was examined for stationarity.

The consensus tree obtained in the former analysis was visualized with FigTree v1.4.2 (Rambaut 2014).

The distances between the taxa and the monophyly of clades were analyzed by phylogenetic networks analysis (PNA) as implemented in Splits Tree 4.14.2 (Huson and Bryant 2006). The monophyly of the lineages was assessed with the Neighbor-Net (splitstransform = EqualAngle) method (Bryant and Moulton 2004), and the bootstrapping estimate (1,000 runs) was employed to support divisions.

Geometric morphometrics analysis

The geometric morphometrics semilandmark method was applied to capture the overall shape variation of the epipharynx (or labrum) since this structure can provide a detailed survey of the more complicated relationships among the taxa (Tocco et al. 2011; Roggero et al. 2015). On the basis of the former biomolecular and morphological analyses (see above), two main issues were identified. One comprised the overall epipharynx shape variation within the whole dataset to assess the reciprocal relationships among all the taxa. The other comprised a more precise characterization of the shape variation patterns that distinguish *Phalops, Digitonthophagus* and *Onthophagus* 21st group.

The configuration of points (Fig. 2) was chosen to capture the overall shape variation of the epipharynx, and was sampled using tpsDig2 v2.20 (Rohlf 2015a) and tpsUtil v1.64 (Rohlf 2015b). The same points configuration was employed to examine the patterns of shape variation in both datasets (see above) applying the same protocol. This comprised Principal component analysis (a.k.a., Relative warps analysis), Canonical variate analysis and Multivariate tests of significance (Roggero et al. 2013).

Reciprocal relationships among the species were evaluated for both datasets (N_1= 84 and N_2 = 62) using tpsSmall v1.33 (Rohlf 2015c) and tpsRelw v1.54 (Rohlf 2015d). Relative warp values (RWs) and the aligned configurations (AL) were retained for further analyses.

Canonical Variates analysis (CVA) on the RWs values was employed to test the proposed taxa classifications as implemented in IBM© SPSS© Statistics v22 (IBM Corp. 2013). This procedure applied the Malahanobis distance method and the leave-one-out option on the whole dataset of the RWs values to account for 100% of the overall shape variation.

The goodness of group assignations was examined by tpsRegr v1.42 (Rohlf 2015e), employing the aligned configurations gained from the PCA (see above) to test the proposed classifications through a taxa comparison. For the analysis, a design matrix was chosen (Rohlf 2015e) to represent the current experimental design for the study of specimens classification. The significance of the classification was tested by Permutation tests (N reps=100,000) as implemented in tpsRegr.

Results

Taxonomic revision

The species formerly included in *Onthophagus* 21st group are separated as a new genus, *Kurtops* **gen.n.**, that was included in the *Phalops* complex (see online Supplementary Material for further details)

Kurtops **Roggero, Barbero and Palestrini gen.n.**

(Figs 3, 4, 5 and 6)

Type species. *Onthophagus signatus* Fåhraeus, 1857: 304.

Included species. At present, the three species that formerly constituted the *Onthophagus* 21st group (Fåhraeus 1857; Harold 1867; d'Orbigny 1902, 1913) are included in the new genus. A detailed description of the species included in the genus can be found in the online Supplementary Material.

Description. Length 0.50-1.00 cm. Head squared, without horns or laminar extensions, covered by a thick, whitish pubescence; rounded and slightly protrunding genae; small superior portion of eyes. Pronotum covered by thick rasping points, with a long, light yellow pubescence thicker on sides. Marked elytral striae, with points as large as the striae. Pygidium with deep, irregular points, and slightly larger in males. Legs characterized by testaceous femurs, and darker tibiae; fore tibia stouter in males than in females, with an evident tooth only in males.

Epipharynx (Fig. 3). Fore margin only slightly notched, sickle-shaped in *K. caffrarius* and *K. quadraticeps*, more squared in *K. signatus*; corypha constituted by a well-developed tuft of setae; the triangular sclerotized area below the haptomerum almost reaching the coripha, narrow at base in *K. signatus*, and larger in *K. quadraticeps* and *K. caffrarius*; apotormae always present, more or less developed; hollow area below the haptolachus (i.e., the plegmatic area) narrowed (*K. quadraticeps*) or inapparent (*K. caffrarius* and *K. signatus*); reduced and thick pternotormae; very short and rounded laeotorma and the dexiotorma. On the whole, the epipharynx features of *Kurtops* are well-differentiated from those of *Digitonthophagus* and *Phalops* (Fig. 7).

Male genitalia (Figs. 4D-F, 5). Aedeagus parameres rounded and slightly tapering at apex, with a well-developed inward expansion (triangular in *K. signatus*, and beak-shaped in *K. quadraticeps* and *K. caffrarius*). Phallobase twice as long as the parameres, slightly inward curved. Well-differentiated endophallus sclerites, but lamella copulatrix absent; accessory lamellae well developed, sharing a similar pattern to *Digitonthophagus* and *Phalops* ones (Fig. 8); FLP always well-developed, the apical part expanded, rounded and less sclerotized, carrying many small teeth, and the basal part expanded into a lamina more or less developed, but always well sclerotized; FLP carrying also a lateral part (here named EC) triangular shaped and well-developed; conspicuous BSC sclerite near the base of the FLP sclerite; C-shaped and tightly connected A and SA sclerites positioned laterally to FLP; SRP sclerite present, more or less developed.

Female genitalia (Fig. 6). The females are known only for *K. quadraticeps* and *K. signatus*, that show a similar pattern, analogous to that already seen in *Phalops* and *Digitonthophagus* (Fig. 9). Moderately sclerotized infundibular wall, triangular-shaped in *K. quadraticeps*, and more clearly mushroom-shaped in *K. signatus*. Receptaculum seminis well sclerotized, slender, elongate, tapering to the sharp apex, with the glandular tube opening very near the point of insertion of the infundibular tube.

Etimology. The new genus was named after the characteristically rounded pronotum, employing the Greek word kurtos that means convex.

Distribution. The genus is known from the whole Southern African subregion (Fig. 10).

Remarks. According to the results of biomolecular and morphological analyses, these species constitute a distinct monophyletic taxon that is closely related to *Digitonthophagus* and *Phalops*. They were thus removed from *Onthophagus* and raised to generic level. Although these three species show similar features, they can be easily identified from each other. *Kurtops caffrarius* differs greatly from *K. signatus* on the basis of the size and general appearance. It differs from *K. quadraticeps* essentially by the pronotum, that is evenly covered by granulate small points in *K. caffrarius*, and with granulate larger points which are smaller only on hind central half in *K. quadraticeps*. The rasping points and the simple points are mixed in the *K. signatus* pronotum. The yellowish ochreous elytra in *K. quadraticeps* and *K. signatus* carry darker patches, while they are evenly ochreous in *K. caffrarius*.

The epipharynx (Fig. 3) fore margin is rounded in *K. quadraticeps* and *K. caffrarius*, squared in *K. signatus*; the proplegmatium is narrow in *K. signatus*, but thicker in the two other species; the apotormae are linear shaped in *K. signatus*, more developed and almost reaching the proplegmatium in *K. caffrarius*, while in *K. quadraticeps* lengthens beyond the proplegmatium line.

In males the parameres apices (Fig. 4) are triangular-shaped in *K. signatus*, hook-shaped in *K. quadraticeps* and *K. caffrarius*, although they are far more developed in the latter species; the endophallus lamellae are very differently shaped in the three species (Fig. 5).

In females (Fig. 6) the infundibular wall in *K. signatus* and *K. quadraticeps* is very differently shaped, in accordance with what has already been seen in *Phalops* and *Digitonthopagus* (Barbero et al. 2003).

Molecular analysis

The pairwise distance matrix is shown in Table 3 (Supplementary Material). Distances were mostly >0.1 except for *O. ovatus*/*O. coenobita*, *O. nuchicornis*/*O. medius* and *O. ovatus*/*O. nuchicornis* that had a distance value <0.1. These lower distance values were found only within some Palearctic *Onthophagus*, and are likely due to recent speciation events. Two major groups were clearly identified. In one, pairwise distance values were always > 0.6-0.8, corresponding to a group comprising only *Onthophagus* species. The second group comprises *Onthophgus interstitialis* and other genera.

The ML trees showed two major clades. One comprised *Phalops* + *Digitonthophagus* + *Kurtops*. The second was divided into two further clades. One includes the *Onthophagus s.l.*+*O. interstitialis* species while the other comprised *Euonthophagus flavimargo* + *Onthophagus depressus*. Small differences were shown among the species within each clade, but the support values were homogeneous in all the computed trees. Both SH-like aLRT and bootstrap gave congruent support values for the major clades.

High bootstrap (100%) and SH-like aLRT (1) values were shown for separation of the *Onthophagus* clade in the ML tree (TN93 BIC = 8793.309, Fig. 11), although the support values were frequently lower within the clade. This result was expected since only a fraction of the many *Onthophagus* species were considered in the present research, thus the intrageneric relationships surely could not be fully elucidated. The position of *O. interstitialis*, *O. depressus* and *E. flavimargo* could not be resolved, although the results showed closer relationships to *Onthophagus s.l.* than to the *Phalops* + *Digitonthophagus* + *Kurtops* clade (the latter one showing bootstrap = 27%, but SH-like aLRT = 0.775). Within the last clade, the support values were high for *Digitonthophagus* and *Kurtops* **gen.n.**, but for *Phalops* the intrageneric relationships were not fully supported. The particularly low value shown for *Phalops* may depend on the fact that only two out of the 38 known species have been used in the analysis, and the two species belong to two distinct clades within *Phalops*.

The tree generated by phylogenetic networks analysis (see online Supplementary Material) showed significant recomputed fit values (fit = 98.744, LS fit 99.983 and stress = 0.013). Significant bootstrap values of 100% were shown for the two major clades and all included species groups (see online Supplementary Material).

Morphological analysis

Phylogenetic analysis

The first heuristic search performed on the matrix of unordered and equal weight characters (Table 2) generated six trees (length = 111, CI = 0.594, HI = 0.405, RI = 0.750, RC = 0.445, not shown here). Successive weighting analysis was then applied to generate a single tree (Fig. 12A, length = 49.130, CI = 0.775, HI = 0.224, RI = 0.887, and RC = 0.687) where two major clades were identified. In the first clade, two groups were distinguished, one including *Onthophagus bituberculatus and O. depressus*, the other comprising *Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.** In the second major clade all the other species were included.

Implicit Enumeration and the Traditional Search (with Implied Weighting set to ON) as implemented in TNT gave analogous results. By both methods a single tree (length = 115, CI = 0.595, RI = 0.750) was produced, that was identical to the one from maximum parsimony analysis in PAUP. The standard bootstrap, jackknife and symmetric resampling methods generated congruent support values at a generic level, with the average group support equal to 48.1, 51.5 and 51.7 respectively. The support statistics from TNT were congruent to the ones from the Bootstrap in PAUP (see Fig. 12A).

The majority rule 50% consensus tree (Fig. 12B) produced by the Bayesian Inference method was not fully resolved. While the genera were well-defined, having a good credibility value, the reciprocal relationships among the genera were not clearly established, and the nodes were collapsed. The chain swap information for the two runs generated equal results for proportion of successful state exchanges between chains. TRACER confirmed the correctness of the Bayesian Inference by the analysis of the statistics of the two runs.

The resulting network splits tree (Fig. 12C) from the Phylogenetic Networks analysis (NeighborNet Equal Angle algorithm) had a recomputed fit = 95.18, and LS fit = 99.62. The Resampling by the bootstrap method confirmed the proposed groups, as already shown in the former analyses. The support values of the genera were marked onto the tree (Fig. 12C). The close relationships among *Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.** were assessed, as well as those within the *Onthophagus* species. *Euonthophagus flavimargo* is isolated from the other species, and not related to the *Onthophagus* species (see Moretto 2009 for further details). Also *Onthophagus bituberculatus and O. depressus* constituted a distinct clade secluded from the others, and these species are currently under review based on the results obtained by this research.

Geometric morphometrics analysis

In the analysis on the whole dataset of Onthophagini, the correlation value of the tangent distances against the Procrustes distances obtained by tpsSmall was 1.000, thus the amount of variation in shape in the present dataset was small enough to permit the subsequent GM analysis.

In the principal component analysis (PCA, as implemented in tpsRelw), forty out of the forty-six obtained RWs were enough to account for 100% of the overall shape variation, thus the last six RWs were discarded from the following analysis. Each of the first four RWs gave a percent value of explained variance greater than 5%. These RWs accounted together for almost 75% of the overall shape variation, being approximately 50% of the overall shape variation represented by the two first RWs (plots not shown here).

The deformation grids of the RWs 1-4 axes (Fig. 13) were examined separately, and marked differences were displayed. In RW_1 the main changes involved the fore margin, that can be more or less notched, the width of the proplegmatium, the length of the triangular sclerotized medial area below the haptomerum, and the more or less accentuate curvature of the chaetopariae. RW_2 represents variation in the fore margin together with marked differences in development of the crepis. RW_3 accounted mainly for the shape variation of the hollow area which is located at the base of the anterior epitorma, and can be more or less expanded. Variations of the fore margin, and length of the medial sclerotized area were summarized by RW_4.

Due to the great number of RWs obtained from the PCA, these variables cannot be (as usual) examined in pairs by means of graphics to furnish a full representation of the overall shape variation. The taxa classification was tested for all the variables that gave together 100% of explained variance (i.e., forty RWs) using CVA.

CVA analysis of variation in shape of the epipharynx defined four well-separated groups (Fig. 14A) that were consistent with taxonomic classification (Fig. 12). High goodness of fit was confirmed by cross validation (98.8%, Table 4, Supplementary Material). Figure 14A shows that the species of *Onthophagus* group 21 are more closely related to *Digitonthophagus* and *Phalops* than to *Onthophagus s.l.* Figure 14B shows that group 21 is, nevertheless, separate from *Digitonthophagus* and *Phalops* thus justifying its status as the new genus *Kurtops*.

In the tpsRegr analysis, the Multivariate tests of significance gave significant values (Hotelling-Lawley trace = 25.469, $F_{(184\,,\,130.0)} = 4.499$, $p < 0.0001$). The Generalized Goodall F-test also gave a significant result (F = 11.1477, df = 184, 3634, and p = 0.0000). The results of the Permutation tests, based on 100.000 replications, are in agreement with the former findings (see above), being the percent of Goodall F values ≥ observed equal to the significant value of 0.001% (small percentages imply significance).

Also for the second analysis, the amount of variation in shape obtained by tpsSmall was small enough (1.000) to permit the subsequent GM analysis of the *Phalops* complex dataset.

From the principal component analysis (PCA), forty out of the forty-six obtained RWs accounted for 100% of the overall shape variation, thus the last six RWs were discarded from the following analysis. About 54% of the overall shape variation was represented by the two first RWs, and each of the first four RWs gave a percent value of explained variance greater than 5%, accounting together for almost 72% of the overall shape variation. The three genera are clearly distinguishable in the scatterplot of RW 1 and 2 (the plots of the RWs in pairs are not showed here).

The CVA testing the taxa classification at generic level (Table 5, Supplementary Material) gave 100.0% of cases correctly classified for *Phalops*, *Digitonthophagus* and *Kurtops*, and 98.4% after the cross validation. In the CV 1 and 2 plot (Fig. 14B), the three genera were well-differentiated, *Digitonthophagus* and *Kurtops* **gen.n.** seemingly being more closely related among themselves than to *Phalops*.

The Multivariate tests of significance by the tpsRegr analysis gave a significant value of the Hotelling-Lawley trace (60.374, $F_{(184\,,\,42.0)} = 3.445$, $p < 0.0001$). The Generalized Goodall F-test gave a significant result (F = 6.6993, df = 184, 2622, and p = 0.0000). Also, the results of the Permutation tests based on 100,000 replications were significant, with the percent of Goodall F values ≥ observed equal to the significant value of 0.001%.

Discussion

The study was aimed mainly at evaluating the taxonomic position of the 21st *Onthophagus* species-group within the Onthophagini. The present findings indicate that the group does not belong in *Onthophagus s.l,* and must be raised to generic rank as *Kurtops* **gen.n**. Furthermore, it was confirmed that *Onthophagus* as currently defined is not a monophyletic taxon, which concurs with recent findings (Monaghan et al. 2007; Wirta et al. 2008; Mlambo et al. 2015).

When looking at the results of both biomolecular and morphological analyses of *Kurtops* **gen.n**., *Phalops and Digitonthophagus*, there was a homogenous pattern that was not evident in the *Onthophagus s.l* species, thus excluding any relationship between the former three genera and the latter genus. Therefore, it was hypothesized that the three genera might constitute a distinct taxonomic group separate from the other Onthophagini.

Herein, we recommend to include *Kurtops* **gen.n.,** *Phalops* and *Digitonthophagus* into a *Phalops* complex of genera distinct from Onthophagus in order to further mark its separation from the other Onthophagini, as was previously suggested for the *Serrophorus* complex (Tarasov and Kabakov 2010; Tarasov and Solodovnikov 2011), until the systematic position of all the taxa currently within this tribe (especially, the *Onthophagus*) can be fully elucidated (see online Supplementary Material for further details).

High pairwise distance values from the COI sequence identified two main distinct groups, one including the *Onthophagus* species and the other comprising the *Phalops* complex together with *Euonthophagus flavimargo*, *Onthophagus depressus* and *O. interstitialis*, An ancient separation was accounted for in the taxa from the Afrotropical Region, whilst the Palearctic *Onthophagus* species showed lower pairwise values, thus indicating a more recent speciation than the Afrotropical taxa. The seclusion of *Onthophagus s.l.* was also confirmed by other biomolecular analyses (ML and PNA). It is noteworthy that the *Phalops* complex constituted a distinct clade from all the other taxa, in both trees. Furthermore, *O. interstitialis* was never linked to the *Onthophagus* species, confirming it as a separate clade whose taxonomic status must surely be reviewed.

Consistent results were obtained from the morphological phylogenetic analyses, confirming the presence of two distinct clades for the *Onthophagus s.l.* and the *Phalops* complex, although ostensibly also *Euonthophagus flavimargo* and *Onthophagus bituberculatus + O. depressus* were identified as distinct clades. The hypothesis of a far greater taxonomic complexity than is currently believed within the Onthophagini was thus corroborated.

The highlighted differentiation of these taxa was also confirmed by the geometric morphometrics analysis, in which the epipharynx was adequate by itself to identify the same four groups already classified by the phylogenetic analyses founded on both morphological and (partly) biomolecular data.

To summarize the results, it was found that *Digitonthophagus*, *Phalops* and *Kurtops* **gen.n.** are both closely related, and are characterized by extremely differentiated external features, quite different epipharynx (Figs. 3 and 7) and markedly similar genitalia (Figs. 4-6 and 8-9) patterns (See below for a thorough review of the *Phalops* complex, with an in-depth discussion of the epipharyngeal and genitalic features).

The combination of biomolecular and morphological analyses has definitely contributed in solving the question of the taxonomic position of the three species formerly included in d'Orbigny 21st group, confirming again that *Onthophagus s.l.* is not a monophyletic taxon. Past and present results clearly indicate the need for an urgent review of the classification of each group currently included in this genus, both to define in detail the phylogenetic relationships among these Afrotropical taxa, and to increase the systematic delineation of the whole Onthophagini tribe.

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Tables

Table 1. List of the COI sequences with the GENBANK accession number.

species	**GenBank accession**	**distribution**	**acronym**
Digitonthophagus gazella (Fabricius, 1787)	EF188213.1	Worldwide	GAZ_1
Digitonthophagus gazella (Fabricius, 1787)	EF188212.1	Worldwide	GAZ_2
Euonthophagus flavimargo (d'Orbigny, 1902)	EF188209.1	Afrotropical	FLA_1
Euonthophagus flavimargo (d'Orbigny, 1902)	EF188210.1	Afrotropical	FLA_2
Onthophagus depressus (Harold, 1871)	EF188207.1	Afrotropical	DEP
Onthophagus coenobita (Herbst, 1783)	KM445555	Palearctic	COE
Onthophagus illyricus (Scopoli, 1763)	HQ954129	Palearctic	ILL_1
Onthophagus illyricus (Scopoli, 1763)	KM450900	Palearctic	ILL_2
Onthophagus interstitialis (Fahraeus, 1857)	JN804624.1	Afrotropical	INT_1
Onthophagus interstitialis (Fahraeus, 1857)	JN804625.1	Afrotropical	INT_2
Onthophagus medius (Kugelann, 1792)	KM447997	Palearctic	MED
Onthophagus nigriventris d'Orbigny, 1905	EU162459.1	Afrotropical	NIG
Onthophagus nuchicornis (Linnaeus, 1758)	HQ954131	Palearctic	NUC
Onthophagus ovatus (Linnaeus, 1767)	HQ954130	Palearctic	OVA
Onthophagus signatus (Fahraeus, 1857)	EF188216.1	Afrotropical	SIG_1
Onthophagus signatus (Fahraeus, 1857)	EF188215.1	Afrotropical	SIG_2
Phalops ardea (Klug, 1855)	AY131935.1	Afrotropical	ARD
Phalops rufosignatus Lansberge, 1885	JN804662.1	Afrotropical	RUF_1
Phalops rufosignatus Lansberge, 1885	JN804660.1	Afrotropical	RUF_2
Phalops rufosignatus Lansberge, 1885	JN804661.1	Afrotropical	RUF_3
Serrophorus seniculus (Fabricius, 1781)	EF188225.1	Oriental	SEN

Table 2. Matrix of the 35 morphological characters used in the phylogenetic analysis.

species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
S. seniculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. gazella	0	1	0	1	1	0	0	2	0	0	0	0	0	1	0	1	0	0
D. bonasus	0	1	0	1	1	0	0	2	0	0	0	0	0	1	0	1	0	0
P. ardea	0	2	0	1	1	0	0	2	0	2	0	0	0	0	1	0	2	0
P. rufosignatus	1	2	0	1	0	0	0	2	0	2	0	0	0	0	1	0	2	0
P. wittei	1	2	0	1	0	0	0	2	0	2	0	0	0	0	1	0	2	0
K. signatus	1	1	1	0	1	2	1	1	1	1	2	1	1	2	0	1	0	0
K. quadraticeps	2	1	1	0	0	0	0	1	1	1	2	1	0	0	0	1	0	1
K. caffrarius	2	1	1	0	0	0	0	1	1	1	2	1	0	0	0	1	0	1
E. flavimargo	1	4	1	1	2	1	2	1	1	3	2	1	2	0	0	1	1	0
O. nuchicornis	1	0	2	1	1	0	1	2	1	0	0	0	0	1	0	0	0	0
O. coenobita	1	1	2	1	1	0	1	2	0	0	0	1	0	1	0	0	1	0
O. illyricus	1	2	0	1	1	0	1	1	0	0	1	1	0	0	0	0	1	0
O. medius	1	0	2	1	1	0	1	2	0	0	0	1	0	1	0	0	1	0
O. nigriventris	1	0	0	1	0	0	1	2	0	0	1	1	0	1	0	1	0	0
O. ovatus	1	1	2	1	1	2	1	2	1	0	0	0	1	1	0	0	1	0
O. bituberculatus	0	3	1	1	2	1	1	0	0	0	0	2	0	2	0	0	1	1
O. depressus	0	1	1	1	2	1	1	0	0	0	0	2	1	2	0	0	1	1

species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
S. seniculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. gazella	0	0	0	0	0	1	-	0	0	2	1	1	0	2	2	0	2
D. bonasus	0	0	0	0	0	1	-	0	0	2	1	1	0	2	2	0	2
P. ardea	0	1	1	0	0	1	-	0	0	2	1	1	0	2	2	0	1
P. rufosignatus	1	1	1	0	0	1	-	0	0	2	1	1	0	2	2	0	1
P. wittei	1	1	1	0	0	1	-	0	0	2	1	1	0	2	2	0	1
K. signatus	1	0	0	0	2	1	-	0	0	2	1	1	0	2	2	0	2
K. quadraticeps	1	1	0	0	2	1	-	0	0	2	1	1	0	2	2	0	2
K. caffrarius	1	1	0	0	2	1	-	0	0	2	1	1	?	?	?	?	?
E. flavimargo	1	0	0	0	1	0	1	1	1	0	0	0	3	3	0	1	0
O. nuchicornis	1	0	0	0	1	0	2	0	0	1	0	0	1	1	0	1	0
O. coenobita	1	0	0	0	1	0	2	0	0	1	0	0	1	1	0	1	2
O. illyricus	1	0	0	1	1	0	0	1	0	4	0	0	1	0	0	2	1
O. medius	1	0	0	0	1	0	2	0	0	1	0	0	1	0	0	1	0
O. nigriventris	2	0	0	1	1	0	0	1	0	4	0	0	1	0	0	2	1
O. ovatus	1	0	0	0	1	0	2	1	1	1	0	0	1	1	0	2	2
O. bituberculatus	1	1	0	0	3	1	-	0	0	3	0	2	2	2	1	0	2
O. depressus	1	1	0	0	3	1	-	0	0	3	0	2	2	2	1	0	2

Figures

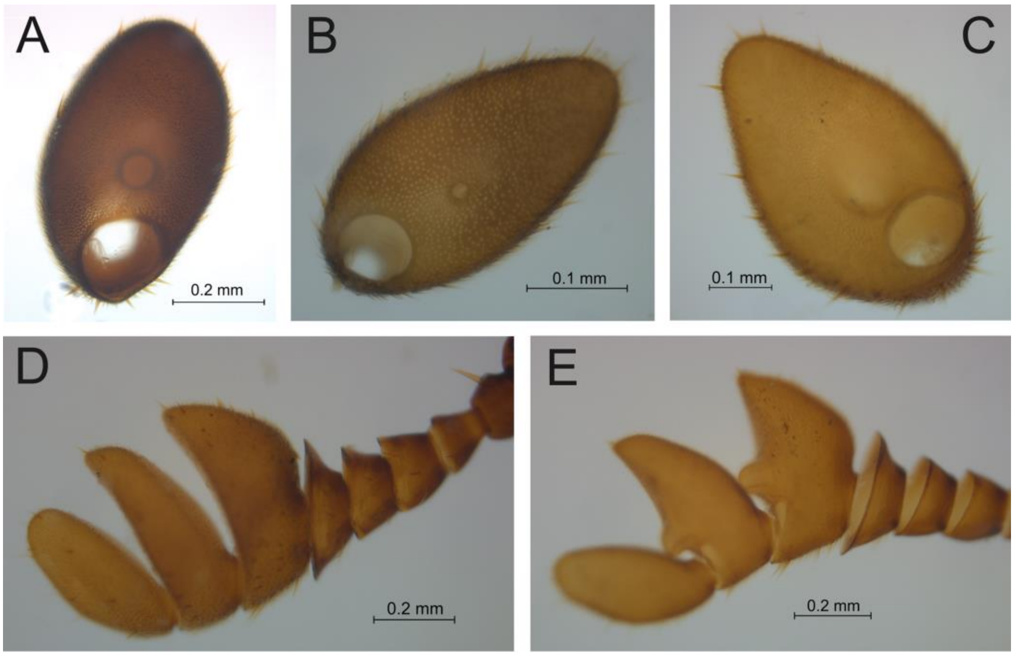

Figure 1. Antennal scape, central cavity of: A) *Phalops ardea*; B) *Kurtops signatus*; C) *Digitonthophagus gazella*; D-E) Different expansions of the central part is shown in two antennae of *Digitonthophagus gazella*.

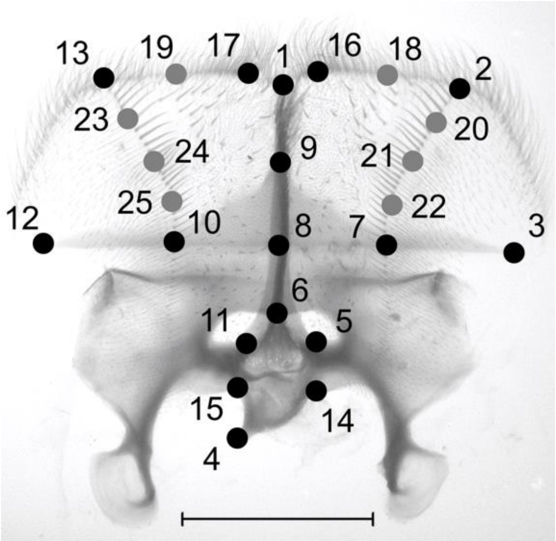

Figure 2. Points configuration for the geometric morphometrics analysis of the epipharynx, with the landmarks marked in black and the semilandmarks in dark grey. Scalebar = 0.5 mm.

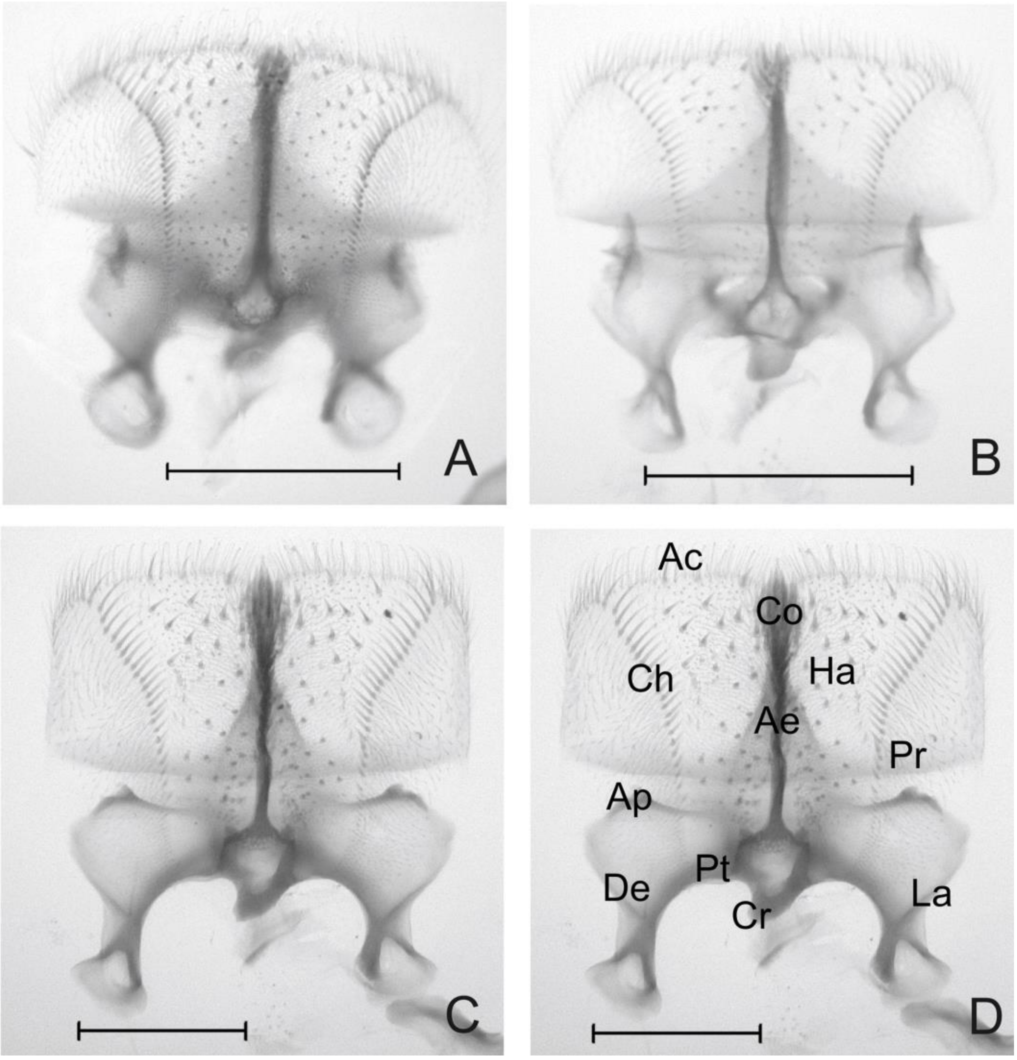

Figure 3. Epipharinx of A) *Kurtops caffrarius* (scalebar = 0.5 mm); B) *K. quadraticeps* (scalebar = 0.5 mm); C) *K. signatus* (scalebar = 0.2 mm); D) Scheme of the various parts of the epipharynx: Ac = Acropariae; Co = Coripha; Ha = Haptomerum; Ch = Chaetopariae; Ae = Anterior epitorma; Pr = Proplegmatium; Ap = Apotormae; Pt = Pternotormae; Cr = Crepis; De = Dexiotorma; La = Laeotorma.

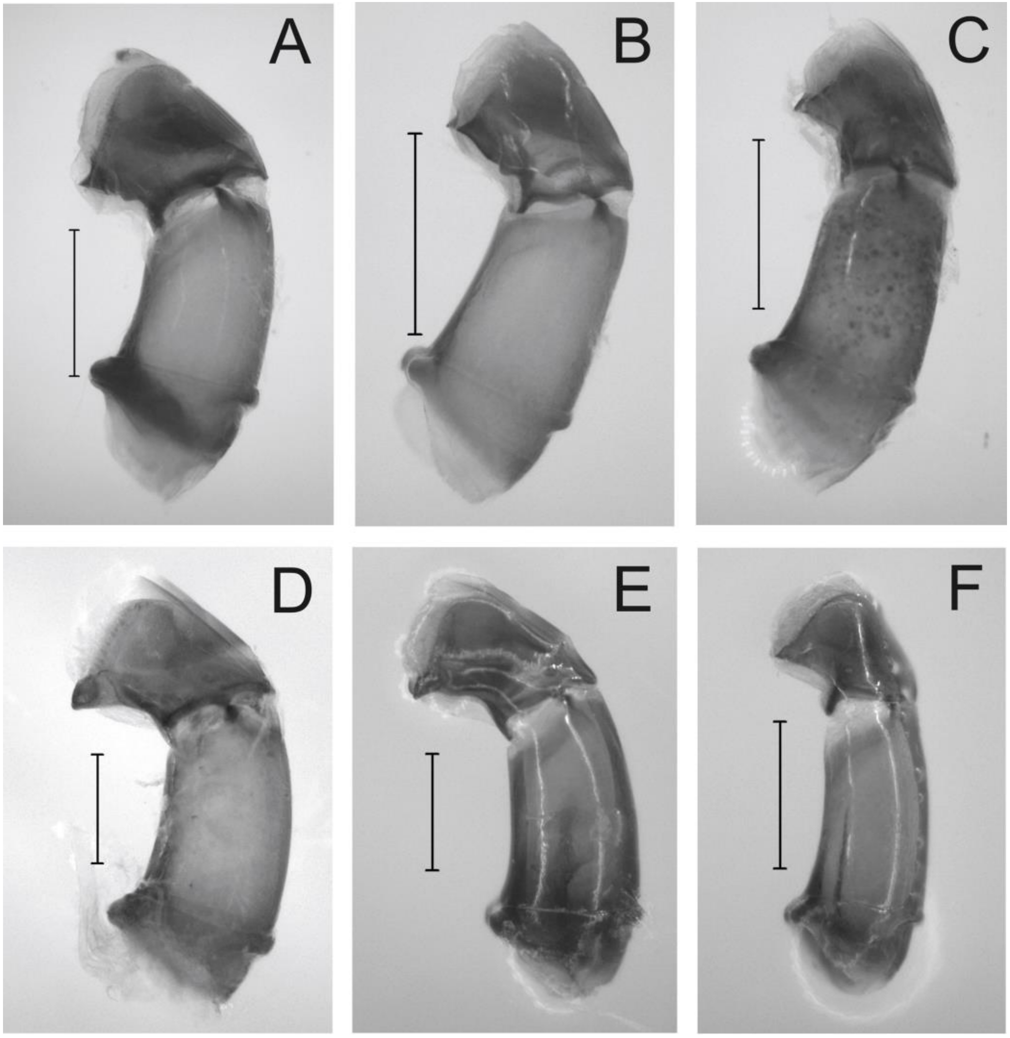

Figure 4. Aedeagus of A) *Digitonthophagus bonasus* (scalebar = 1.0 mm); B) *D. gazella* (scalebar = 1.0 mm); C) *Phalops ardea* (scalebar = 1.0 mm), D) *Kurtops caffrarius* (scalebar = 0.5 mm); E) *K. quadraticeps* (scalebar = 0.5 mm); F) *K. signatus* (scalebar = 0.5 mm).

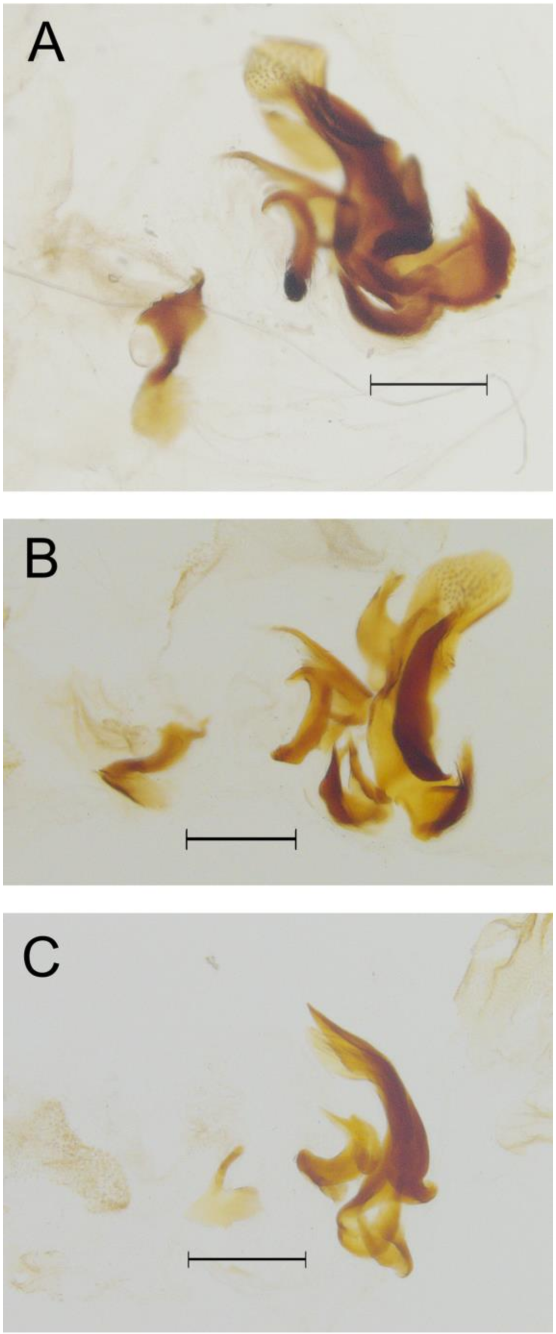

Figure 5. The endophallus sclerites of A) *Kurtops caffrarius*; B) *K. quadraticeps*; C) *K. signatus*. Scalebar = 0.2 mm.

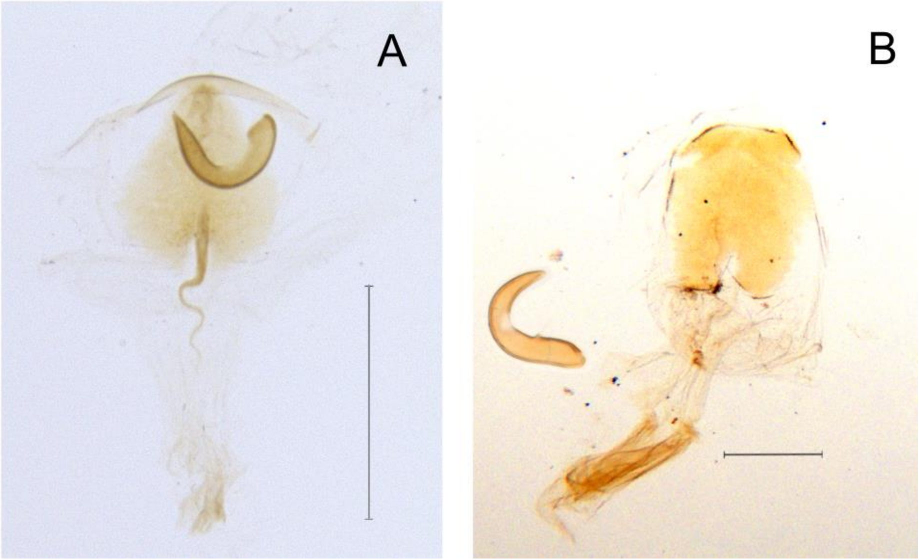

Figure 6. Vagina and receptaculum seminis of A) *Kurtops quadraticeps*, scalebar = 0.5 mm; B) *K. signatus*, scalebar = 0.2 mm.

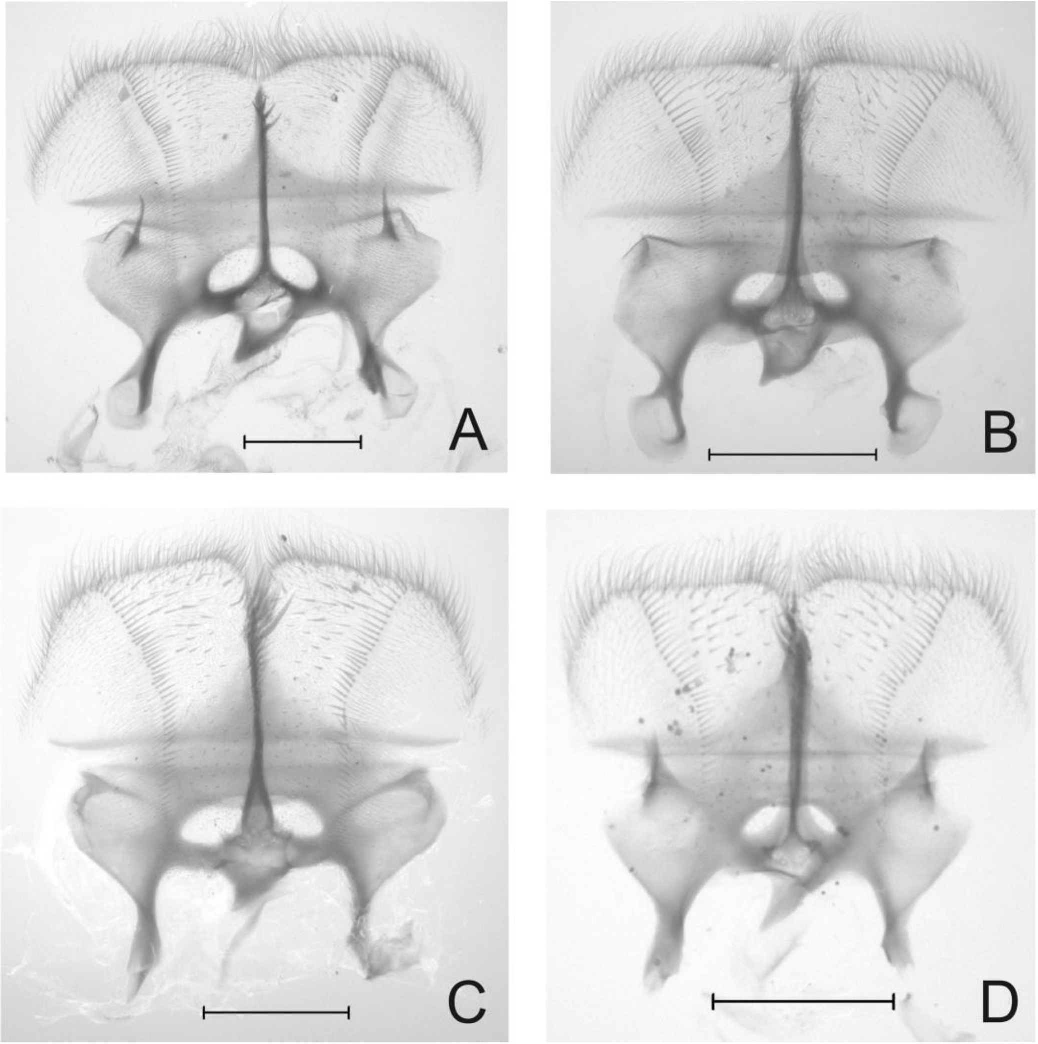

Figure 7. Epipharinx of A) *Digitonthophagus bonasus*; B) *D. gazella*; C) *Phalops ardea*; D) *P. wittei*. Scalebars = 0.5 mm.

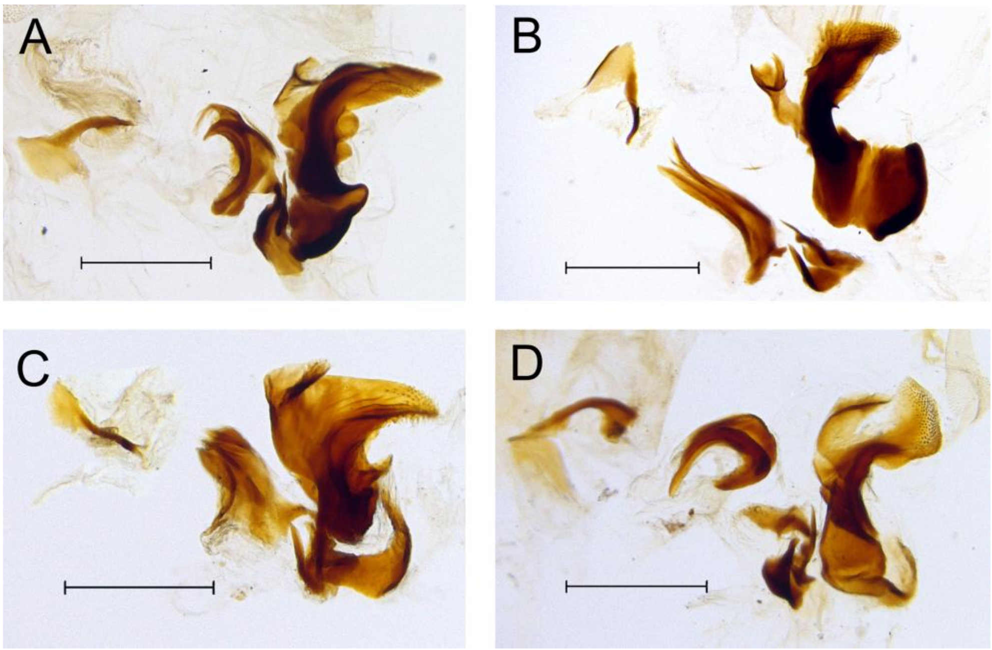

Figure 8. The endophallus sclerites of A) *Digitonthophagus bonasus*; B) *D. gazella*; C) *Phalops ardea*; D) *P. wittei*. Scalebar = 0.5 mm.

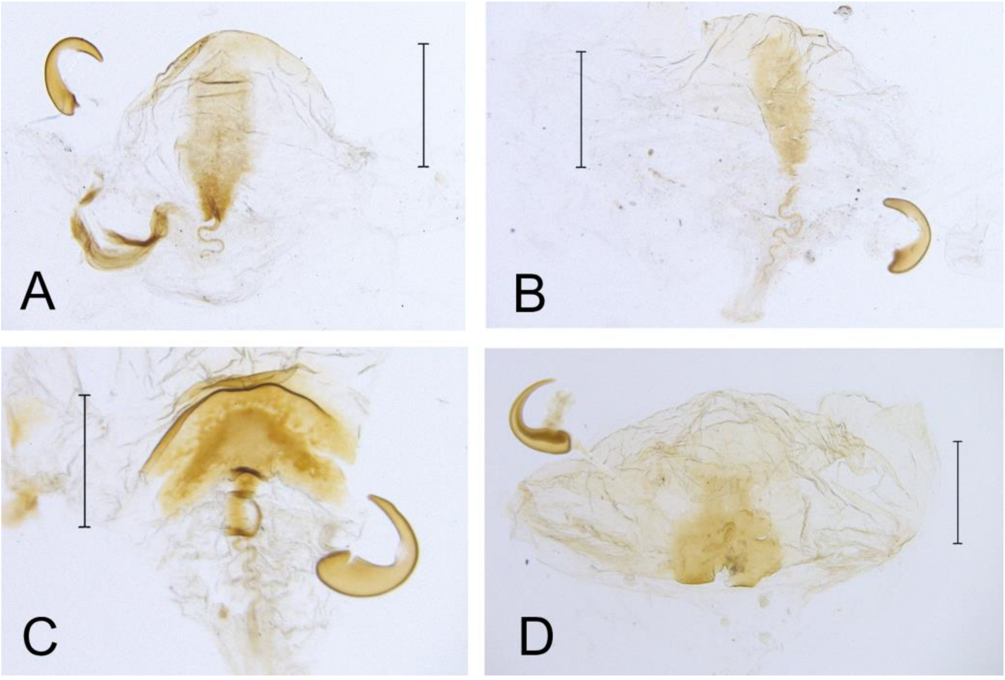

Figure 9. Vagina and receptaculum seminis of A) *Digitonthophagus bonasus*; B) *D. gazella*; C) *Phalops ardea*; D) *P. wittei*. Scalebar = 0.5 mm.

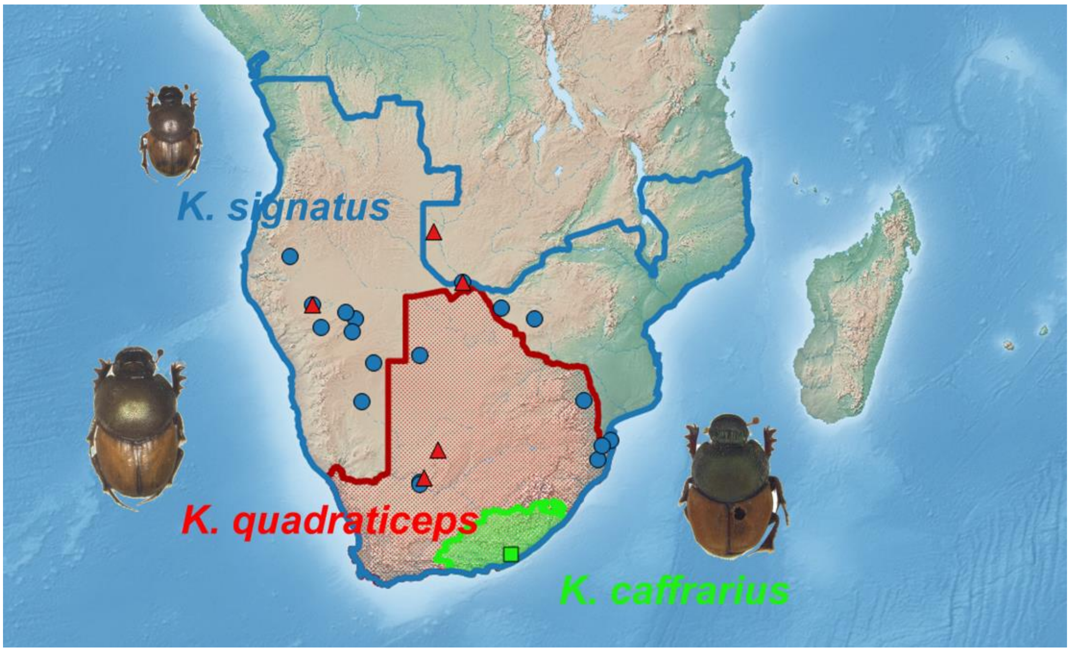

Fig. 10. Distribution map and photos of *Kurtops caffrarius* (green), *K. quadraticeps* (red) and *K. signatus* (blue).

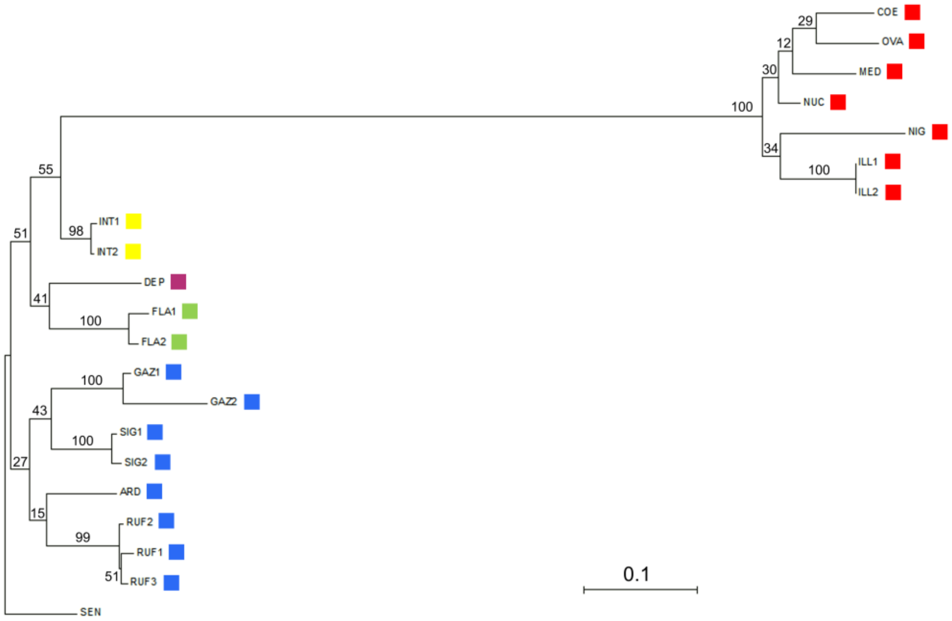

Fig. 11. Maximum Likelihood tree from TN93 method (uniform rates) showing the bootstrap support values on branches. On the tree, *Onthophagus s.l.* are marked in red, *O. depressus* in purple, *O. interstitalis* in yellow, *Euonthophagus flavimargo* in green, and *Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.** in blue. The acronyms are the same as in Table 1: SEN = *Serrophorus seniculus*; GAZ = *Digitonthophagus gazella*; SIG = *Kurtops signatus*; FLA = *Euonthophagus flavimargo;* DEP = *Onthophagus depressus*; COE = *O. coenobita*; ILL = *O. illyricus*; INT = *O. interstitialis*; MED = *O. medius*; NIG = *O. nigriventris*; NUC = *O. nuchicornis*; OVA = *O. ovatus*; ARD = *Phalops ardea*, RUF = *P. rufosignatus*.

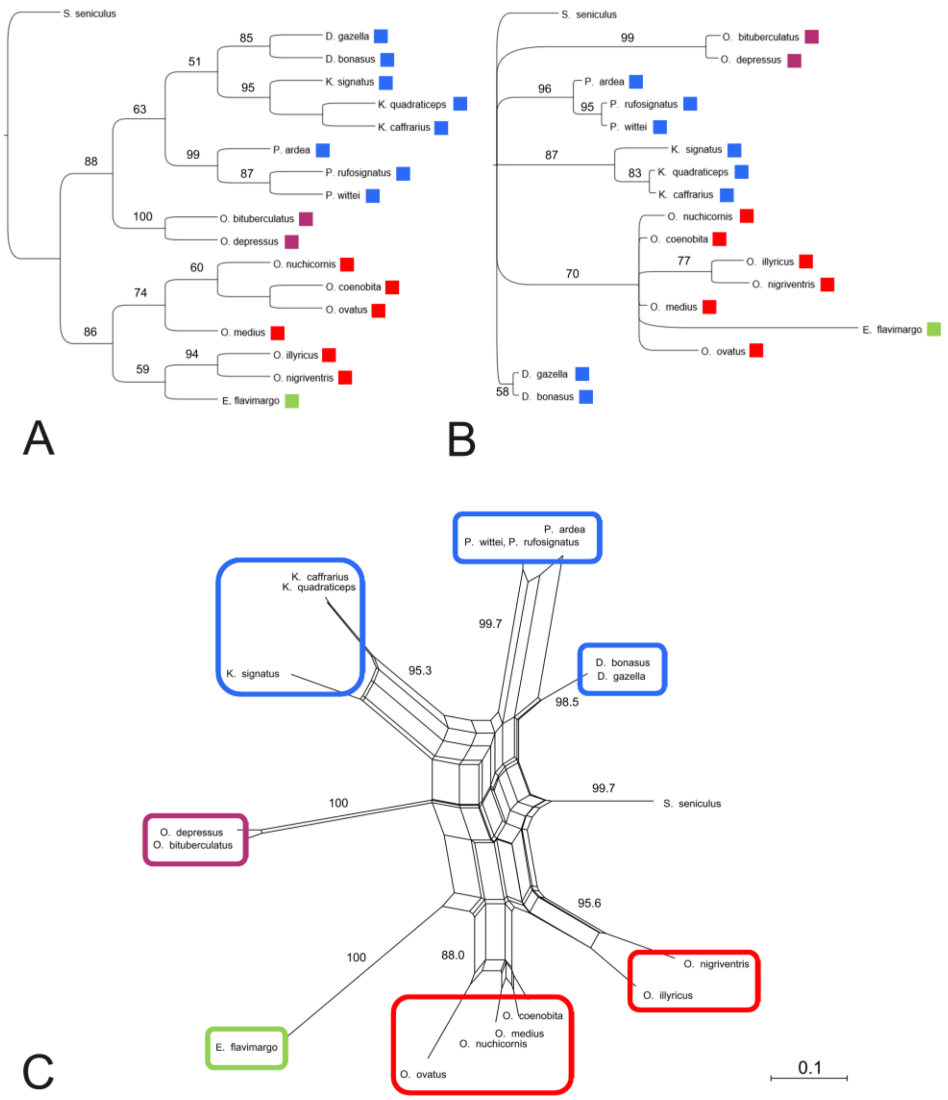

Fig. 12. A) The single tree obtained from maximum parsimony analysis with successive weighting option (Length = 49,130, CI = 0.775). The Bootstrap support values (majority rule 50%) from PAUP are shown above the branches, the resampling from TNT (bootstrap standard, symmetric resampling, and jackknife respectively) gave analogous results (not shown here); B) 50% majority rule consensus tree from Bayesian inference analysis, with the support values shown on branches; C) splits tree by neighbor-net method, with the bootstrap support values for each group shown on branches. In each tree *Onthophagus* are marked in red, *Euonthophagus flavimargo* in green, *Onthophagus bituberculaus* and *O. depressus* in purple, and *Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.** in blue.

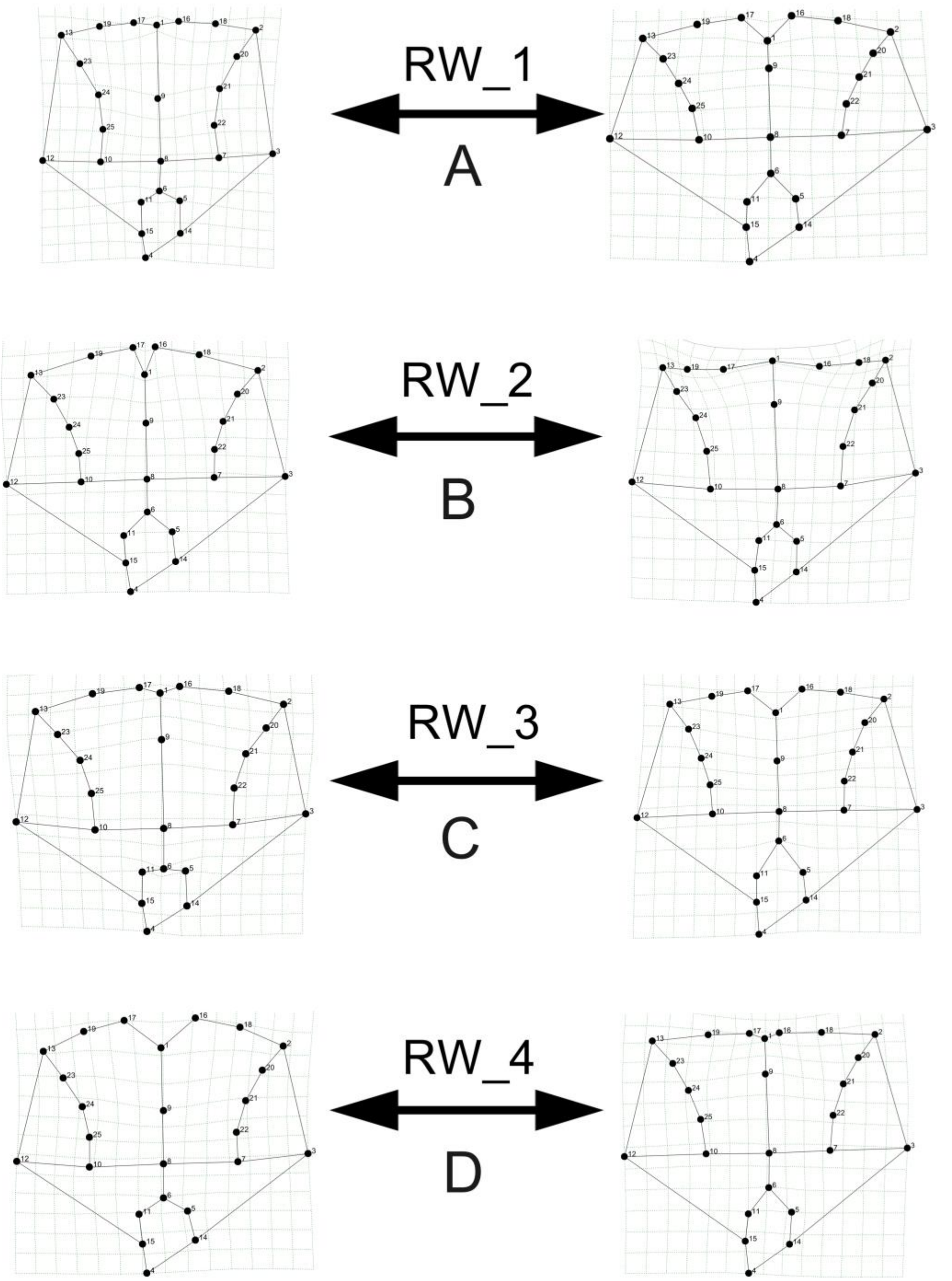

Fig. 13. The extreme deformation grids obtained by each axis of the RWs 1-4, that have percent values of explained variance greater than 5%, namely A) RW_1 = 37.08%, B) RW_2 = 16.81%, C) RW_3 = 11.92%, and D) RW_4 = 9.43%.

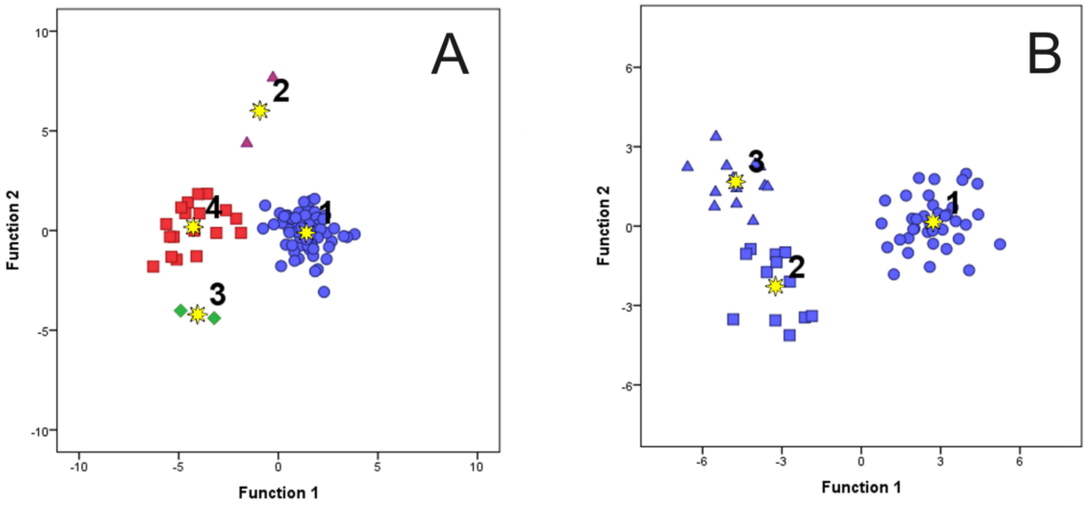

Fig. 14. CVA ordination plots derived from analysis of morphometric data for the epipharynx in which yellow stars represent group centroids. A. Four groups defined for 20 species of Onthophagini: (1) *Phalops*, *Digitonthophagus* and *Kurtops* (blue circles); (2) *Onthophagus bituberculatus* and *O. depressus* (purple triangles); (3) *Euonthophagus flavimargo* (green rhombus); (4) *Onthophagus s.l.* (red squares). B) Three groups defined for genera of the *Phalops* complex (1) *Phalops* (circles); (2) *Digitonthophagus* (squares); (3) *Kurtops* **gen.n.** (triangles).

Supplementary Material

Supplementary material 1 - The endophallus sclerites in Onthophagini

The homologies among the various parts that constitute the extremely complicated endophallus sclerites have been recently highlighted by Tarasov and Solodovnikov (2011) for many Onthophagini. Besides, in *Phalops* and *Digitonthophagus* only the fronto-lateral peripheral (FLP) and the superior right peripheral (SRP) sclerites were definitely recognized, the other sclerites being marked as “unknown” since they were extremely different from those of other taxa examined (Tarasov and Solodovnikov 2011). Subsequently, the homologies of the sclerites within Scarabaeinae were evaluated and assessed by Tarasov and Génier (2015), but neither of the two taxa were included in the analysis. The basal semicircular (BSC), the axial (A) and subaxial (SA) sclerites of some Scarabaeinae may be considered homologous to the “unknown” ones of both *Phalops* and *Digitonthophagus* (Tarasov and Solodovnikov 2011), but also to those of *Kurtops* **gen.n.**

While the BSC sclerite was clearly identifiable as a distinct entity in the examined species, the A and SA sclerites, as hypothesized by Tarasov and Genier (2015), cannot be differentiated in these taxa and must be considered as a single entity. The “unknown” sclerite marked in light yellow by Tarasov and Solodovnikov (2011) cannot be considered as separate from FLP, as is clearly shown in the examined taxa (*Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.**), and is here named EC (external claw) of FLP.

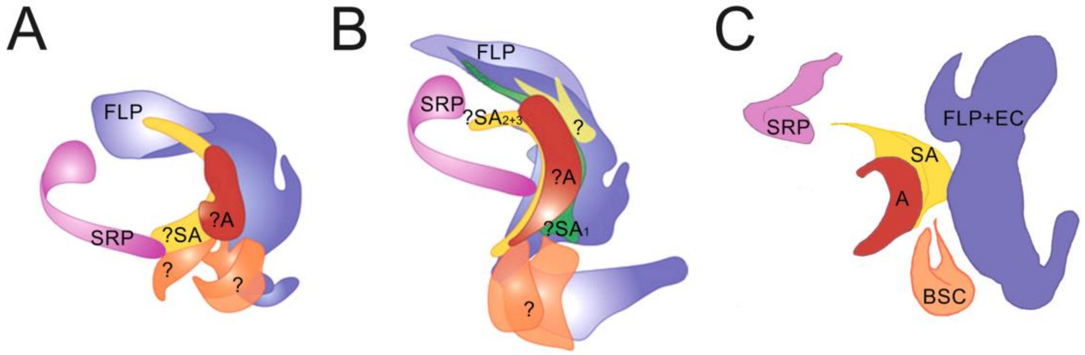

Figure A1. The scheme of the endophallus sclerites of the three genera: A) *Phalops laminifrons*, B) *Digitonthophagus bonasus* (both modified from Tarasov & Solodovnikov 2011), and C) *Kurtops quadraticeps*. The acronyms of the different parts were reported on the figures, and correspond to FLP = fronto-lateral peripheral sclerite, SRP = superior right peripheral sclerite, A = axial sclerite, SA = subaxial sclerite, and BSC = basal semicircular sclerite.

References

Tarasov, S.I. & Génier, F. (2015) Innovative Bayesian and Parsimony Phylogeny of Dung Beetles (Coleoptera, Scarabaeidae, Scarabaeinae) Enhanced by Ontology-Based Partitioning of Morphological Characters. *PlosOne*, *10*, e0116671. doi:10.1371/journal.pone.0116671.

Tarasov, S.I & Solodovnikov, A.Y. (2011) Phylogenetic analyses reveal reliable morphological markers to classify mega-diversity in Onthophagini dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). *Cladistics*, *27*, 1–39.

Supplementary material 2 - Characters list

1. Epipharynx, the fore half in front of the proplegmatium: (0) subtrapezoidal; (1) subrectangular; (2) sickle-shaped.

2. Epipharynx, the fore margin: (0) rectilinear; (1) only slightly sinuate in the middle; (2) deeply and narrowly notched; (3) largely notched; (4) convex.

3. Epipharynx, corypha: (0) absent, only a row of few, sparse and long setae is present along the anterior epitorma; (1) present, constituted by a thick tuft of short setae; (2) present, constituted by short and thin setae.

4. Epipharynx, anterior epitorma: (0) never reaching the fore margin; (1) reaching the fore margin.

5. Epipharynx, anterior epitorma: (0) thick; (1) narrow; (2) very narrow.

6. Epipharynx, the fore triangular sclerotized area of haptomerum: (0) large and lowered; (1) narrow and lowered; (2) narrow and elongate.

7. Epipharynx, proplegmatium position: (0) in the anterior third of the epipharynx; (1) in the medial part of epipharynx surface; (2) in the posterior third of the epipharynx.

8. Epipharynx, chaetopariae: (0) subrectilinear; (1) angulate; (2) arched.

9. Epipharynx, apex of the crepis left turned and: (0) sharp; (1) blunt.

10. Epipharynx, laeotorma and dexiotorma distal part: (0) medium length, with the insertion to mandibles area drop-like; (1) very short and markedly rounded at level of insertion to mandibles; (2) very thin and often elongate, with the insertion to mandibles very narrow; (3) short and spatulate.

11. Epipharynx, pternotormae: (0) short and thick; (1) longer and narrower; (2) greatly reduced.

12. Mentum, fore margin: (0) a large and rounded notch; (1) deeply and triangular notched; (2) a large and triangular notch.

13. Mentum, the basal margin: (0) triangular notched; (1) sinuate; (2) rectilinear.

14. Head, clypeus fore margin: (0) not-incised; (1) only feebly sinuate; (2) deeply V-notched.

15. Head, genae: (0) not especially developed; (1) protruding.

16. Pronotum, on the whole: (0) oval; (1) rounded.

17. Pronotum hind margin: (0) angulate; (1) rounded; (2) straight.

18. Pronotum, fore angles: (0) more or less developed, blunt and always rectilinear, facing forward; (1) well-developed, sharp, and outward turned.

19. Legs, fore tibia: (0) markedly dimorphic in the two sexes, being narrow and inward arched in male; (1) almost identical in both sexes, but showing differences in the tooth shape; (2) showing no sexual dimorphism.

20. Elytra, 7th stria: (0) sinuate; (1) rectilinear.

21. Elytra, 8th stria: (0) absent; (1) present but incomplete, and distally fused to 7th one.

22. Male genitalia, phallobase/parameres ratio: (0) reaching almost 2:1; (1) about 1:1.

23. Male genitalia, parameres: (0) quadrangular symmetrical, carrying laminar expansions ventrally, the apices rounded, with a digitiform expansion subapically; (1) simple, symmetrical, without laminar expansion on ventral side, the apices sharp but short, largely triangular-shaped; (2) arched, the apices large and sharp, carrying a laminar, rounded expansion subapically; (3) triangular-shaped ventrally, pointed at apex, and flat apically.

24. Male genitalia, lamella copulatrix: (0) present; (1) absent.

25. Male genitalia, lamella copulatrix present and : (0) constituted by a globular expansion and a rectilinear part, comma-shaped; (1) well-developed, globose, C-shaped; (2) cupoliform, almost globose, well-sclerotized.

26. Male genitalia, endophallus carrying apically: (0) many small diffusely-arranged scales, sometimes almost effaced; (1) diffusely-arranged scales, and two well-defined, ventral areas with more thickened scales; reduced scales, but a large transversal ridge with well-developed scales.

27. Male genitalia, raspula: (0) absent; (1) present.

28. Male genitalia, FLP (= fronto-lateral peripheral) sclerite: (0) laminar, double, with projections encircling SA+A (= subaxial + axial) sclerite; (1) band-shaped, encircling the SA+A; (2) large, well-developed, with evident expansions apically and basally; (3) linked to SA+A; (4) well sclerotized, and pluridigitate.

29. Male genitalia, SA+A sclerites: (0) rod-shaped, usually separate; (1) C-shaped, connected.

30. Male genitalia, BSC (= basal semicircular) sclerite: (0) absent, (1) present, well-developed; (2) present, very reduced.

31. Female genitalia, infundibular wall: (0) carrying a large longitudinal sclerotization subrectangular or mushroom-shaped; (1) supported by a thin, "arched" sclerotization; (2) with a sinuate, asymmetrical and folded sclerotization; (3) complex sclerotization, with two pillar-shaped lateral sclerotizations and a key-hole central sclerotization.

32. Female genitalia, vagina: (0) enlarged; (1) elongate and wrinkled; (2) rounded; (3) extremely narrow and elongate.

33. Female genitalia, infundibular tube: (0) very sclerotized, orthogonal to infundibular wall; (1) non sclerotized, lowered at floccular level; (2) sigmoidal, placed below the well-developed sclerotization of the infundibular wall.

34. Female genitalia, receptaculum seminis: (0) elongate, tapering to often sharp apex; (1) elongate, subequal on the whole lenght, the apex slightly narrowed, but never sharp; (2) short and tough, rounded at apex.

35. Female genitalia, receptaculum seminis at base: (0) cone-shaped, carrying a sclerotized portion on infundibular tube; (1) gently rounded; (2) truncated.

Supplementary material 3 – Further molecular results

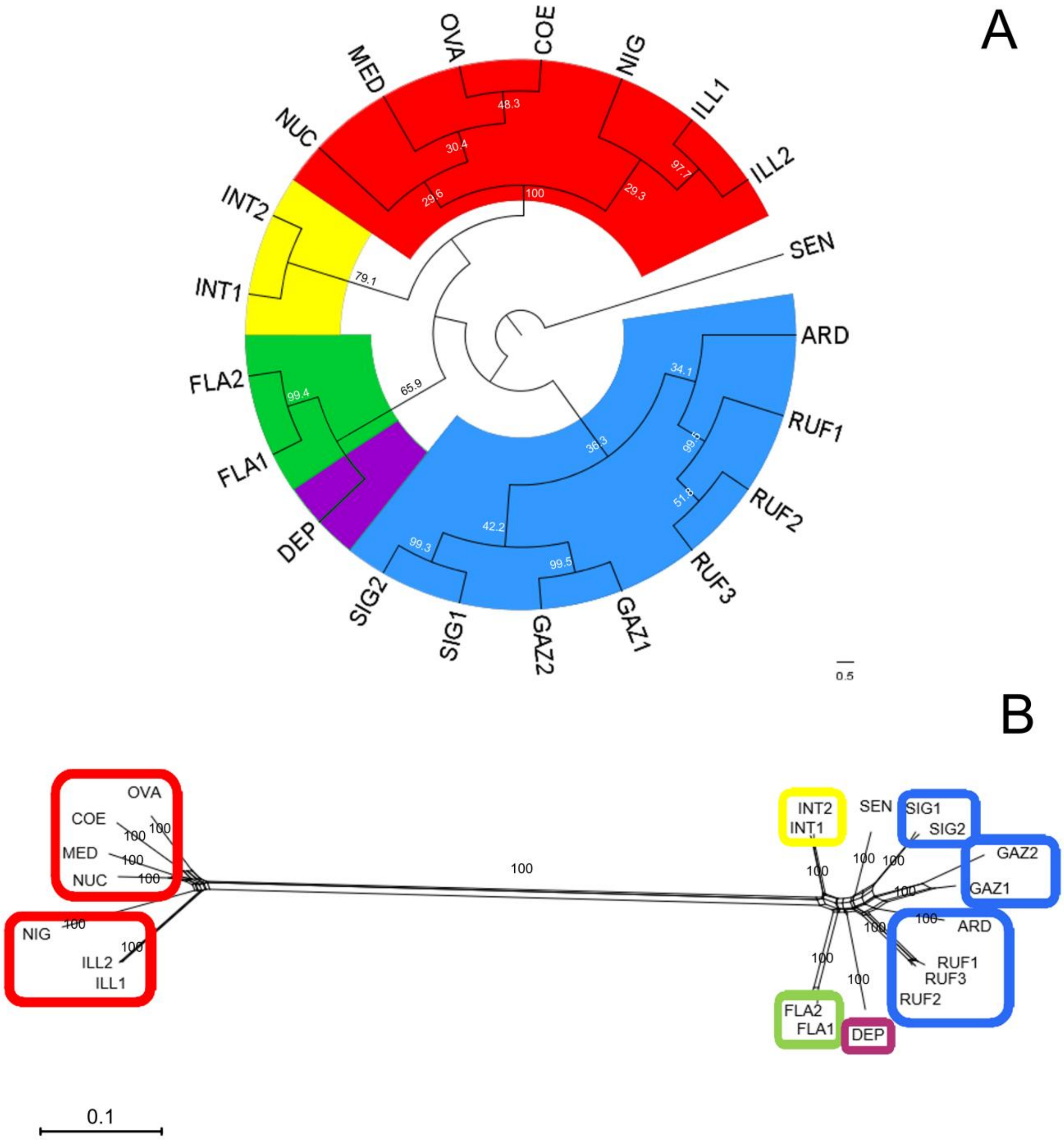

Figure A3. A) Maximum Likelihood tree from TN93 method (uniform rates) showing on branches non-parametric bootstrap support values, similar to the SH like aLRT values (not shown). B) Splits tree by neighbor-net method, with the bootstrap support values for each taxon showed on branches. On each tree, *Onthophagus s.l.* are marked in red, *Euonthophagus flavimargo* in green, *O. depressus* in purple, *O. interstitalis* in yellow, and *Phalops*, *Digitonthophagus* and *Kurtops* **gen.n.** in blue. The acronyms are the same than in Table 1: SEN = *Serrophorus seniculus*; GAZ = *Digitonthophagus gazella*; SIG = *Kurtops signatus*; FLA = *Euonthophagus flavimargo;* DEP = *Onthophagus depressus*; COE = *O. coenobita*; ILL = *O. illyricus*; INT = *O. interstitialis*; MED = *O. medius*; NIG = *O. nigriventris*; NUC = *O. nuchicornis*; OVA = *O. ovatus*; ARD = *Phalops ardea*, RUF = *P. rufosignatus*.

Supplementary material 4 - The *Kurtops* species

The species currently included in *Kurtops* **gen.n.** are here described in detail. The figures quoted here are in

Kurtops caffrarius (d'Orbigny, 1902: 171)

(Figs 3A, 4D, 5A)

Type material. 1 male, holotype. SOUTH AFRICA: Caffraria. Muséum National d'Histoire Naturelle (MNHN, Paris, France).

Description. Length 0.92 cm. Head blackish green, covered by a light yellow, thin and long pubescence; clypeus largely rounded, and genae only slightly expanded; vertex and frontal carinae large, well-developed, blade-shaped; thick and dense granules on the whole surface, antennae ochreous. Pronotum markedly rounded, very dark olive green, entirely covered by small, thick and dense granules and a light yellow, thin and long pubescence. Elytra ochreous and opaque, the striae narrow and the interstriae covered by dense, small setigerous points. Pigidium dark brown entirely covered by large and deep setigerous points, and a dense, light yellow, thin and long pubescence. Very dark brown legs and body lower side. Metasternal disc with large, deep, and rade points.

Epipharynx. The fore margin rounded; acropariae and acanthopariae long and thick; acanthopedia covered by a dense pubescence; corypha constituted by a well-developed tuft of setae; chaetopariae angulate, with the setae short and dense; proplegmatium thick; laeotoema and dexiotorma short and stout, pternotormae very small and thick; crepis well-sclerotized and large, with the apex blunt.

Male genitalia. Phallobase of aedeagus twice as long as the paramers, slightly arched, with the diameter subequal on the whole length; symmetrical paramers with a rounded and just a little protruding superior part, the apices large and sharp, slightly hook-shaped, carrying a laminar and rounded expansion subapically. Endophallus entirely lacking a copulatrix lamella, the accessory lamellae well-developed, comprised of various parts (SRP+FLP/EC+SA+A+BSC), similarl to those of *Phalops* and *Digitonthophagus* (Fig. 8).

Distribution. The species is known only from the type locality in SE Eastern Cape province, formerly designed as Kaffraria (Fig. 10).

Remarks. The species at present is known only from a single specimen, the male holotype from Caffraria. The female is unknown.

***Kurtops quadraticeps* (Harold, 1867: 52)**

(Figs 3B, 4E, 5B, 6A)

Type material. SOUTH AFRICA: Orange Free State. Museum für Naturkunde der Humboldt-Universität (ZMHB, Berlin, Germany).

Description. Length 0.60-1.00 cm. Head dark green, covered by a light yellow, thin and long pubescence; clypeus largely rounded, and genae only slightly expanded; vertex and frontal carinae large, well-developed, blade-shaped; thick and dense granules on the whole surface, antennal scape reddish brown, lamellae ochreous. Pronotum markedly rounded, dark green, entirely covered by a light yellow, thin and long pubescence, with small, thick and dense rasping points reducing from the fore to hind margin, and larger points with smaller granules in the hind central half. Elytra yellowish ochreous often carrying brown and elongate patches more or less developed till covering almost the whole surface, the striae narrow and the interstriae covered by equally spaced, small setigerous points. Pigidium blackish brown, entirely covered by large and deep setigerous points, and a dense, light yellow, thin and long pubescence. Very dark brown legs and body lower side. Metasternal disc with large, deep, and scattered points.

Epipharynx. The fore margin rounded; acropariae and acanthopariae long and thick; acanthopedia covered by a dense pubescence; corypha constituted by a well-developed tuft of setae; chaetopariae angulate, with the setae short and dense; proplegmatium thick; laeotoema and dexiotorma short and stout, pternotormae very small and thick; crepis squared and large.

Male genitalia. Phallobase of aedeagus twice as long as the paramers, slightly arched, with the diameter subequal along the whole length; symmetrical paramers with a rounded and just a little protruding superior part, the apices small and sharp, slightly hook-shaped, carrying a laminar and rounded expansion very near the apices. Endophallus entirely lacking a copulatrix lamella, the accessory lamellae well-developed, comprised of various parts (SRP+FLP/EC+SA+A+BSC), showing the same general pattern to those *Phalops* and *Digitonthophagus* (Fig. 8).

Female genitalia. Infundibular wall triangular-shaped, with the basal part rounded; infundibular tube well-sclerotized, plurisinuate and tapering distally. Receptaculum seminis large, C-shaped, the apex sharp, almost entirely sclerotized, the proximal part to infundibulum shorter than the distal one.

Distribution. The species is known from South Africa (where is diffusely reported from Orange Free state, Cape Colony, Natal, Western Cape [Twee Rivieren]), and Botswana (Fig. 10).

Remarks. It is noteworthy that (unlike *K. caffrarius*) this species has a very wide distribution and is known throughout Southern Africa.

Kurtops signatus **(Fåhraeus, 1857: 304)**

(Figs 3C, 4F, 5C, 6B)

Type material. MOZAMBIQUE: Limpopo river. Naturhistoriska Riksmuseet (NHRS, Stockholm, Sweden).

Synonymy. *O. junodi* d'Orbigny, 1902: 223. Muséum National d'Histoire Naturelle (MNHN, Paris, France).

Description. Length 0.50-0.60 cm. Head shiny black, covered by a rade, light yellow, thin and long pubescence; clypeus deeply V-notched in the middle, and genae only slightly expanded; vertex and frontal carinae large, well-developed, blade-shaped; dense setigerous points on the whole surface, antennae reddish brown. Pronotum rounded, shiny black, with thick and dense setigerous granulate points mixed with smaller simple points and a light yellow, short and thin pubescence. Elytra ochreous with black dots, the striae as large as the striae points, and the interstriae covered by 2 rows of small setigerous points. Pigidium shiny black, covered by superficial points mixed with smaller ones. Very dark brown body lower side and legs, except the ochreous femura. Metasternal disc with large, deep, and scattered points.

Epipharynx. The fore margin squared; acropariae long and thick; acanthopariae short and thin; acanthopedia covered by a pubescence short and evenly distributed; corypha comprising a well-developed tuft of setae; chaetopariae angulate, with the setae long in the anterior half, far shorter in the posterior half; proplegmatium very thin and slightly arched; laeotoema and dexiotorma short and stout, pternotormae very small and thick; short crepis with the apex blunt.

Male genitalia. Phallobase of aedeagus more than twice the length of the paramers, slightly arched, with the diameter subequal along the whole length; symmetrical paramers with a rounded and just a little protruding superior part, the apices large and sharp, subtriangular, the ventral laminar expansion almost not apparent. Endophallus entirely lacking a copulatrix lamella, the accessory lamellae well-developed, comprised of various parts (SRP+FLP/EC+SA+A+BSC), similar to the model already evidenced in *Phalops* and *Digitonthophagus* species (Fig. 8).

Female genitalia. Infundibular wall mushroom-shaped, with the basal part far more developed that the apical part; infundibular tube plurisinuate. Receptaculum seminis large, J-shaped, the apex sharp, almost entirely sclerotized, the proximal part to infundibulum longer than the distal one.

Distribution. The species is known from Angola, Botswana, Mozambique, Namibia, South Africa, and Zimbabwe (Fig. 10).

Remarks. *O.junodi* d’Orbigny, 1902 (from Mozambique) was synonymized to *O. signatus* by d’Orbigny (1913).

References

d’Orbigny, H. (1913) Synopsis des Onthophagides d'Afrique. *Annales de la Société Entomologique de France*, *82*, 1–742.

Supplementary material 5 - The*Phalops* complex

The comparison of *Kurtops* **gen.n.**, with three species, *Phalops* Erichson, with 38 species (Barbero et al. 2003; Genier 2013), and *Digitonthophagus* (Balthasar) with two species (Balthasar 1959, 1963; Zunino 1981) led to the identification of the *Phalops* complex in accord to that already suggested for the *Serrophorus* complex (Tarasov and Kabakov 2010; Tarasov and Solodovnikov 2011).

These three genera are characterized by extremely differentiated external features that are very useful as identification characters. The evident sexual dimorphism present in *Digitonthophagus* and *Phalops* is reduced to the variation of the fore tibiae in *Kurtops*. The male head carries more or less developed horns in *Digitonthophagus*, and laminar projections in *Phalops*, but is unarmed in *Kurtops*. The pronotum in *Phalops* and *Kurtops* has a dense granulation on the whole surface, while in *Digitonthophagus* it is smooth with sparse, large simple points (*D. gazella*) or few granulate points (*D. bonasus*). The pronotum hind margin is straight only in *Phalops*, and the pubescence is far thicker and longer in *Kurtops* than in the two other genera. The 8th elytral stria is absent and the 7th stria sinuate in *Digitonthophagus* and *Kurtops*, while in *Phalops* the 8th stria is distally fused to 7th stria, that is rectilinear.

Also the epipharynx (Figs. 3 and 7) allows to clearly distinguish these taxa (see also the results of the geometric morphometrics analysis above for more details), e.g. the fore margin is more deeply notched in *Phalops* and *Digitonthophagus* than in *Kurtops*, the laeotorma and dexiotorma markedly differ in the three genera, and the apotormae are characteristically more or less developed in the three genera.

These genera share instead a highly similar pattern for both male and female genitalia, that confirms the marked proximity among them. In males, the aedeagus is characterized by short paramers (Fig. 4); in the endophallus the lamella copulatrix (LC) is absent (while in *Onthophagus s.l.* is always present), and the accessory sclerites (FLP, SRP, BSC, and A+SA) are conspicuous, and show an analogous and very characteristic developmental model in the three genera (Figs 5 and 8). In *Onthophagus*, the accessory sclerites features are markedly different from those of the *Phalops* complex, never being as developed. Furthermore, the A+SA sclerites are usually straight and not C-shaped, the BSC sclerite is not present, and the FLP sclerite usually encircles the others (Tarasov and Solodovnikov 2011).

In females, the infundibular wall of the vagina is sub-rectangular or mushroom-shaped, and always well-sclerotized, the infundibulum is short and plurisinuate, and the receptaculum seminis is usually elongate, tapering to a sharp apex, with a very reduced non-sclerotized medial area (Figs 6 and 9). In the *Onthophagus* species here examined, the infundibular wall support is usually constituted by a narrow and (more or less) H-shaped sclerotization, the infundibular tube is well-sclerotized and C-shaped, and the receptaculum seminis has an even diameter along the whole length, the apex rounded, and a very large non-sclerotized medial area.

On the whole, the *Phalops* complex has a worldwide distribution. Its original distribution extends in Palearctic (*Phalops* and *Digitonthophagus*), Afrotropical (*Phalops*, *Kurtops* and *Digitonthophagus*) and Oriental (*Phalops* and *Digitonthophagus*) regions, but was also introduced in Nearctic, Neothopical and Australian regions (only *D. gazella*).

The genus *Phalops* was described by Erichson in 1843 (see Barbero et al. 2003 for further details), and its taxonomic status is not disputed at present. Balthasar (1959:464) described *Digitonthophagus* as a subgenus of *Onthophagus*, with *D. bonasus* (Fabricius, 1775) as type species of the taxon, furnishing later (Balthasar 1963) the list of the 20 species originally included in the taxon. The author remarked that the majority of the *Digitonthophagus* species had an Oriental distribution, and only two were located in the Eastern Palearctic region. Furthermore, according to Balthasar's observations (1959), it was also very likely that some Afrotropical species would have to be included in this taxon. Subsequently, Zunino (1981) raised *Digitonthophagus* to a generic level, including only two out of the 20 species: the Afrotropical *Digitonthophagus gazella* (Fabricius, 1787) having now a worldwide distribution, and the Oriental *D. bonasus* (Fabricius, 1775). The remaining Balthasar's *Digitonthophagus* species were later assigned to five different subgenera within *Onthophagus* (Ochi 2003a, 2003b), that were subsequently included in the *Serrophorus* complex (Tarasov and Kabakov 2010; Tarasov and Solodovnikov 2011).

References

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```
#NEXUS

[ File saved by NDE version 0.5.0 ]

BEGIN TAXA;
      DIMENSIONS NTAX=18;
      TAXLABELS
            'S. seniculus'
            'D. gazella'
            'D. bonasus'
            'P. ardea'
            'P. rufosignatus'
            'P. wittei'
            'K. signatus'
            'K. quadraticeps'
            'K. caffrarius'
            'O. nuchicornis'
            'O. coenobita'
            'O. illyricus'
            'O. medius'
            'E. flavimargo'
            'O. nigriventris'
            'O. ovatus'
            'O. bituberculatus'
            'O. depressus'
            ;
ENDBLOCK;

BEGIN CHARACTERS;
      DIMENSIONS NCHAR=35;
      FORMAT DATATYPE=STANDARD MISSING=? GAP=- SYMBOLS="01234";
      CHARLABELS
            [1] 'Epipharynx, the fore half till the prophlegmatium'
            [2] 'Epipharynx, the fore margin'
            [3] 'Epipharynx, corypha'
            [4] 'Epipharynx, anterior epitorma'
            [5] 'Epipharynx, anterior epitorma'
            [6] 'Epipharynx, the fore triangular sclerotized area of 
haptomerum'
            [7] 'Epipharynx, proplegmatium placed'
            [8] 'Epipharynx, chaetopariae'
            [9] 'Epipharynx, apex of the crepis left turned and'
            [10] 'Epipharynx, laeotorma and dexiotorma distal part'
            [11] 'Epipharynx, pternotormae'
            [12] 'Mentum, fore margin'
            [13] 'Mentum, the basal margin'
            [14] 'Head, clypeus fore margin'
            [15] 'Head, genae'
            [16] 'Pronotum, on the whole'
            [17] 'Pronotum hind margin'
            [18] 'Pronotum, fore angles'
            [19] 'Legs, fore tibia'
            [20] 'Elytra, 7th stria'
            [21] 'Elytra, 8th stria'
            [22] 'Male genitalia, phallobase/parameres ratio'
            [23] 'Male genitalia, paramers'
            [24] 'Male genitalia, lamella copulatrix'
            [25] 'Male genitalia, lamella copulatrix present and'
            [26] 'Male genitalia, endophallus carrying apically'
```

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            [27] 'Male genitalia, raspula'
            [28] 'Male genitalia, FLP sclerite'
            [29] 'Male genitalia, SA+A sclerites'
            [30] 'Male genitalia, BSC sclerite'
            [31] 'Female genitalia, infundibular wall'
            [32] 'Female genitalia, vagina'
            [33] 'Female genitalia, infundibular tube'
            [34] 'Female genitalia, receptaculum seminis'
            [35] 'Female genitalia, receptaculum seminis at base'
            ;
      STATELABELS
            1
                  'subtrapezoidal'
                  'subrectangular'
                  'sickle-shaped',
            2
                  'rectilinear'
                  'only slightly sinuate in the middle'
                  'deeply and narrowly notched'
                  'largely notched'
                  'convex',
            3
                  'absent, only a row of few and rade large setae is
present along the anterior epitorma'
                  'present, constituted by a thick tuft of short setae'
                  'present, constituted by short and thin setae',
            4
                  'never reaching the fore margin'
                  'reaching the fore margin',
            5
                  'thick'
                  'narrow'
                  'very narrow',
            6
                  'large and lowered'
                  'narrow and lowered'
                  'narrow and elongate',
            7
                  'in the anterior third of the epipharynx'
                  'in the half of epipharynx surface'
                  'in the posterior third of the epipharynx',
            8
                  'subrectilinear'
                  'angulate'
                  'arched',
            9
                  'sharp'
                  'blunt',
            10
                  'medium length, with the insertion to mandibles area
drop-like'
                  'very short and rmarkedly rounded at level of
insertion to mandibles'
                  'very thin and often elongate, with the insertion to
mandibles very narrow'
                  'short and spatulate',
            11
                  'short and thick'
                  'longer and narrower'
```

```
            'greatly reduced',
      12
            'a large and rounded incisure'
            'deeply and triangular notched'
            'a large and triangular incisure',
      13
            'triangular notched'
            'sinuate'
            'rectilinear',
      14
            'not-incised '
            'only feebly sinuate'
            'deeply V-notched',
      15
            'not especially developed'
            'protrunding',
      16
            'ovalar-transversal'
            'rounded',
      17
            'angulate'
            'rounded'
            'straight',
      18
            'more or less developed, blunt and always rectilinear, facing forward'
            'well-developed, sharp, and outward turned',
      19
            'markedly dimorphic in the two sexes, being narrow and inward arched in male'
            'almost identical in both sexes, but showing differences in the tooth shape'
            'showing no sexual dimorphism',
      20
            'sinuate'
            'rectilinear',
      21
            'absent'
            'present but incomplete, and distally fused to 7th one',
      22
            'reaching almost 2:1'
            'about 1:1',
      23
            'quadrangular simmetrical, carrying laminar expansions ventrally, the apices rounded, with a digitiform expansion subapically'
            'simple, symmetrical, without laminar expansion on ventral side, the apices sharp but short, largely triangular-shaped'
            'arched, the apices large and sharp, carrying a laminar, rounded expansion subapically'
            'triangular-shaped ventrally, pointed at apex, and flat apically',
      24
            'present'
            'absent',
      25
            'constituted by a globose expansion and  a rectilinear part, comma shaped'
```

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                  'well-developed, globose, C-shaped'
                  'cupoliform, globose, well-sclerotized',
            26
                  'many small teeth diffused somtimes almost
inapparent'
                  'diffused scales, and two well-defined, ventral areas
with more tickened scales; riduced scales, but a large transversal
bent with well-developed scales',
            27
                  'absent'
                  'present',
            28
                  'laminar, double, with projections encircling SA+A'
                  'band-shaped, encircling the SA+A'
                  'large, well-developed, with evident expansions
apically and basally'
                  'linked to SA+A'
                  'well sclerotized, and pluridigitate',
            29
                  'rod-shaped, usually separate'
                  'C-shaped, connected',
            30
                  'absent'
                  'present, well-developed'
                  'present, very reduced',
            31
                  'carrying a large longitudinal sclerotization
subrectangular or mushroom-shaped'
                  'supported by a thin, "arched" sclerotization'
                  'with a sinuate asymmetrical and folded
sclerotization'
                  'complex sclerotization, with two pillar-shaped
lateral sclerotizations and a key-hole central sclerotization',
            32
                  'transversal '
                  'elongate and wrinkled'
                  'rounded'
                  'extremely narrow and elongate',
            33
                  'very sclerotized, orthogonal to infundibular wall'
                  'non sclerotized, lowered at floccular level'
                  'sigmoidal, placed below the well-developed
sclerotization of the infundibular wall',
            34
                  'tapering to apex, elongate, often the apex sharp'
                  'elongate, subequal on the whole lenght, the apex
slightly narrowed, but never sharp'
                  'short and tough, rounded at apex',
            35
                  'cone-shaped, carrying a sclerotized portion on
infundibular tube'
                  'gently rounded'
                  'truncated',
      ;
      MATRIX
            'S. seniculus'             0000000000 0000000000 0000000000
00000
            'D. gazella'               0101100200 0001010000 0001-00211
02202
```

```
            'D. bonasus'             0101100200 0001010000 0001-00211
02202
            'P. ardea'               0201100202 0000102001 1001-00211
02201
            'P. rufosignatus'        1201000202 0000102011 1001-00211
02201
            'P. wittei'              1201000202 0000102011 1001-00211
02201
            'K. signatus'            1110121111 2112010010 0021-00211
02202
            'K. quadraticeps'        2110000111 2100010111 0021-00211
02202
            'K. caffrarius'          2110000111 2100010111 0021-00211
?????
            'O. nuchicornis'         1021101210 0001000010 0010200100
11010
            'O. coenobita'           1121101200 0101001010 0010200100
11012
            'O. illyricus'           1201101100 1100001010 0110010400
10021
            'O. medius'              1021101200 0101001010 0010200100
10010
            'E. flavimargo'          1411212113 2120011010 0010111000
33010
            'O. nigriventris'        1001001200 1101010020 0110010400
10021
            'O. ovatus'              1121121210 0011001010 0010211100
11022
            'O. bituberculatus'      0311211000 0202001111 0031-00302
22102
            'O. depressus'           0111211000 0212001111 0031-00302
22102
      ;
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ENDBLOCK;

BEGIN NOTES;
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      [Character state comments]

      [Attribute comments]

      [Taxon pictures]

      [Character pictures]

      [Character state pictures]

      [Attribute pictures]
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Table 3. Pairwise distance matrix (overall average value = 0.416), in which estimates of evolutionary divergence between sequences were conducted using the Kimura 2-parameter model. See table 1 for the acronyms.

	SEN	**GAZ_1**	**GAZ_2**	**ARD**	**RUF_1**	**RUF_2**	**RUF_3**	**SIG_1**	**SIG_2**	**NUC**	**COE**	**ILL_1**	**ILL_2**	**MED**	**FLA_1**	**FLA_2**	**NIG**	**OVA**	**INT_1**	**INT_2**	**DEP**
SEN																					
GAZ_1	0,150																				
GAZ_2	0,208	0,081																			
ARD	0,130	0,119	0,186																		
RUF_1	0,128	0,132	0,201	0,125																	
RUF_2	0,123	0,130	0,196	0,125	0,017																
RUF_3	0,123	0,134	0,201	0,130	0,017	0,011															
SIG_1	0,126	0,124	0,175	0,130	0,160	0,148	0,153														
SIG_2	0,128	0,129	0,175	0,142	0,157	0,150	0,155	0,013													
NUC	0,677	0,761	0,814	0,721	0,722	0,700	0,700	0,733	0,699												
COE	0,697	0,752	0,828	0,764	0,741	0,724	0,719	0,735	0,711	0,100											
ILL_1	0,734	0,768	0,844	0,726	0,745	0,712	0,706	0,765	0,746	0,110	0,130										
ILL_2	0,734	0,768	0,844	0,726	0,745	0,712	0,706	0,765	0,746	0,110	0,130	0,000									
MED	0,702	0,795	0,864	0,753	0,759	0,736	0,731	0,764	0,728	0,081	0,117	0,143	0,143								
FLA_1	0,146	0,171	0,221	0,176	0,162	0,153	0,153	0,152	0,155	0,697	0,715	0,732	0,732	0,739							
FLA_2	0,141	0,160	0,226	0,164	0,141	0,141	0,137	0,162	0,164	0,677	0,696	0,706	0,706	0,728	0,026						
NIG	0,781	0,869	0,923	0,795	0,804	0,791	0,785	0,849	0,820	0,145	0,164	0,173	0,173	0,178	0,798	0,769					
OVA	0,677	0,747	0,839	0,718	0,705	0,684	0,674	0,715	0,703	0,091	0,106	0,119	0,119	0,121	0,701	0,676	0,177				
INT_1	0,126	0,169	0,216	0,132	0,148	0,137	0,137	0,127	0,126	0,601	0,657	0,657	0,657	0,653	0,126	0,128	0,732	0,620			
INT_2	0,126	0,164	0,211	0,130	0,146	0,134	0,134	0,127	0,126	0,601	0,653	0,653	0,653	0,653	0,124	0,130	0,743	0,620	0,007		
DEP	0,152	0,163	0,205	0,161	0,156	0,150	0,143	0,173	0,175	0,713	0,751	0,741	0,741	0,754	0,157	0,150	0,807	0,713	0,143	0,136	

Table 4. Results of the first CVA in which the major groups classification was confirmed, since 100.0% of original grouped cases were correctly classified, and after the cross validation the 98.8% of cross-validated grouped cases were correctly classified.

Classification Results

			Predicted Group Membership				
code group			1	2	3	4	Total
Original	Count	1	62	0	0	0	62
		2	0	2	0	0	2
		3	0	0	2	0	2
		4	0	0	0	18	18
	%	1	100,0	0,0	0,0	0,0	100,0
		2	0,0	100,0	0,0	0,0	100,0
		3	0,0	0,0	100,0	0,0	100,0
		4	0,0	0,0	0,0	100,0	100,0
Cross-validated	Count	1	62	0	0	0	62
		2	0	2	0	0	2
		3	0	0	2	0	2
		4	1	0	0	17	18
	%	1	100,0	0,0	0,0	0,0	100,0
		2	0,0	100,0	0,0	0,0	100,0
		3	0,0	0,0	100,0	0,0	100,0
		4	5,6	0,0	0,0	94,4	100,0

Table 5. Results of the second CVA in which the genera classification within the *Phalops* complex was confirmed, since 100.0% of original grouped cases were correctly classified, and after the cross validation the 98.4% of cross-validated grouped cases were correctly classified.

Classification Results

code genus			Predicted Group Membership 1	2	3	Total
Original	Count	1	37	0	0	37
		2	0	12	0	12
		3	0	0	13	13
	%	1	100,0	0,0	0,0	100,0
		2	0,0	100,0	0,0	100,0
		3	0,0	0,0	100,0	100,0
Cross-validated	Count	1	37	0	0	37
		2	0	12	0	12
		3	0	1	12	13
	%	1	100,0	0,0	0,0	100,0
		2	0,0	100,0	0,0	100,0
		3	0,0	7,7	92,3	100,0