Species delimitation by a geometric morphometric analysis within the genus *Pseudoathyreus* and description of a new species (Coleoptera: Scarabaeoidea: Bolboceratidae)

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Abstract
A geometric morphometric analysis, conducted as part of a taxonomic review of the genus *Pseudoathyreus* (Coleoptera: Bolboceratidae), allowed us to highlight the differences within a group of closely related species spread from the Sahel region to India, supporting the traditional morphological approach and confirming the presence of a newly discovered species (*P. zianii* n. sp.).

Keywords: Morphology, biometry, taxonomy, biogeography, arid environments.

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Introduction
According to Král & Batelka (2017) *Pseudoathyreus orientalis* (Laporte de Castelnau, 1840) is the only valid species of *Pseudoathyreus* Howden and Martínez, 1963 (Bolboceratidae: Athyreinae) occurring in Asia. In the framework of a revision (still in progress) of the genus *Pseudoathyreus* we investigated the Asian population and assessed all the available names, *P. frontalis* (Parry, 1845) and *P. flavohirtus* (Walker, 1871), in order to deepen the subject.

*Pseudoathyreus orientalis* is the only Asian species currently recognized as valid: although the type seems to be untraceable, some ancient toptotypical specimens are kept either in the Natural History Museum of London (NHM) or in the Musem National d’Histoire Naturelle of Paris (MNHN), labelled “Bengal” and “Bengale” respectively, that is the type locality explicitly reported in the original description.

*Pseudoathyreus frontalis* was described from NE India (Khasi Hills, Meghalaya) but the type material of this species is completely unknown to the curators of all the museums we contacted. Moreover, the original description (Parry 1845) is very basic and scarcely detailed, and led us to suggest that this species could either be a junior synonym of *P. orientalis* or belong to another genus of bolboceratid beetles. The last hypothesis is more probable because the type locality falls within a moist tropical forest area whereas all other known distribution records for this species and for the other *Pseudoathyreus* species fall within drier habitats (at most arid savannas or semideserts).

*Pseudoathyreus flavohirtus* was described from “Hor Tamanib” in the Red Sea coastal region of Sudan. Type material of this species could be traced neither in NHM nor in the Oxford Museum, where the remains of the Walker’s collection are deposited (Horn & Kahle 1936; Král & Batelka 2017). Moreover, Boucomont (1912) interpreted erroneously the type locality of *P. flavohirtus* as “Arabien”, probably because of the title and the structure of the original publication of Walker (1871), and caused confusion in subsequent authors (Janikova 1998; Carpaneto et al. 2000; Barari 2001; Gillett & Gillett 2005; Král et al. 2006; Nikolajeve et al. 2016; Abdel-Dayem et al. 2017). During our revision, we obtained a specimen referable to this species, from eastern Sudan (Kassala), captured about 390 kms south of the type locality, where the climatic conditions and vegetation type are very similar (White 1983; Linder et al. 2005).

After having examined extensive material from various collections we got the following picture of the situation:

1. two specimens from the western Arabian coast (between Mecca and Medina) very similar to the specimens collected in Kassala, led us to state that *P. flavohirtus* really occurs on both coasts of the Red Sea;
2. two old specimens from Mali and Niger, also referable...
to *P. flavohirtus*, led us to suggest that its distribution extends throughout the Sahel Region;

3. two series of specimens respectively collected in Senegal (Saint-Louis) and Eritrea (Massawa), seem to belong to a same group of species / subspecies that hereafter we name “*P. flavohirtus* complex” and which will be the subject of a future paper;

4. several specimens collected from the eastern Arabian peninsula, southern Iran and southern Pakistan, resulted to be a new species of *Pseudoathyreus*, related to *P. orientalis* but well distinct from it, and will be described in the present paper.

As the morphological traits delimiting the three taxonomic units (*P. orientalis*, *P. flavohirtus* complex and the new species) seem mainly based on the shape of the median ridge of the anterior pronotal border, we performed a geometric morphometric analysis of this structure to support the validity of the traditional taxonomic criteria used in the species delimitation of this genus. Our aim was to get an objective assessment of such morphological characters through the application of a statistical method.

### Material and methods

To find a straightforward delimitation of the three taxonomic units and to define their geographic distribution, we analysed the shape variation of the anterior pronotal border (APB) by a Geometric Morphometric Analysis (GMA), using a set of landmark coordinates. Such biometric approach preserves the shape of the structure, allowing a visual inspection of its actual variation (Mitteroecker et al. 2013). This method was widely used by the entomologists to investigate shape variation at intra- and interspecific level, focusing on different research topics, such as, ecomorphology, evolution, phylogeny, animal weapons (Hernández et al. 2011; Polihronakis 2006; Pretorius et al. 2000; Romiti et al. 2017).

For GMA of the APB, we chose 26 individuals, at least seven for each taxonomic unit (*P. orientalis*, *P. flavohirtus* complex and the new species herein described) (Table 1).

For our analysis we used a set of 25 points (3 landmarks and 22 semi-landmarks), digitized by a single operator using tpsDig2 software ver. 2.17 (Rohlf 2013), plotting semi-landmarks at regular intervals between land-

<table>
<thead>
<tr>
<th>Species</th>
<th>Region (state or province)</th>
<th>Locality</th>
<th>no. samples</th>
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</thead>
<tbody>
<tr>
<td><em>P. flavohirtus</em> complex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ER: Eritrea</td>
<td>Northern Red Sea</td>
<td>Massawa</td>
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<td>SA: Saudi Arabia</td>
<td>Mecca</td>
<td>Turbah</td>
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<tr>
<td>SD: Sudan</td>
<td>Kassala</td>
<td>Kassala</td>
<td>1</td>
</tr>
<tr>
<td>SN: Senegal</td>
<td>Saint-Louis</td>
<td>Richard Toll</td>
<td>4</td>
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<td><em>P. zianii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR: Iran</td>
<td>Hormozgan</td>
<td>Bandar Abbas</td>
<td>1</td>
</tr>
<tr>
<td>IR: Iran</td>
<td>Kerman</td>
<td>Baghuiyeh</td>
<td>1</td>
</tr>
<tr>
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<td>Sistan and Baluchestan</td>
<td>Sarbaz</td>
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</tr>
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<td>Al Wusta</td>
<td>Yalooni</td>
<td>2</td>
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<tr>
<td>SA: Saudi Arabia</td>
<td>Riyadh</td>
<td>Riyadh</td>
<td>1</td>
</tr>
<tr>
<td>AE: United Arab Emirates</td>
<td>Abu Dhabi</td>
<td>Al Ajban</td>
<td>1</td>
</tr>
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<td></td>
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<tr>
<td>IN: India</td>
<td>“Bengale”</td>
<td>unknown</td>
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<tr>
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<td>Abu Road</td>
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<tr>
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<td>Jaisalmer</td>
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<tr>
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<td>Pilani</td>
<td>1</td>
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<td>PK: Pakistan</td>
<td>Waziristan</td>
<td>unknown</td>
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marks (Fig. 1). Next, a generalized Procrustes analysis (GPA) was used to superimpose the configurations of landmarks in all specimens to a common coordinate system, removing the effect of size, position, and orientation. Then, the results were interpreted graphically with thin-plate spline (TPS) deformation grids of the first two principal components (PCs) of the GPA, using ggplot2 package (Wickham 2016) in R software, version 3.5.1 (R Core Team 2014). Additionally we performed a multivariate analysis of variance (MANOVA) and an unweighted pair-group average (UPGMA) cluster analysis using Past version 3.20 (Hammer et al. 2001). MANOVA, considering those PCs that account for 95% of the cumulative variance, was used to test for shape differences among species, proceeding with a Hotelling’s tests for pairwise comparisons if any. UPGMA, with Euclidean distances similarity index, was performed using the first 10 PCs to test whether there is a phylogenetic signal between species and produce a dendrogram of the clusters. The percentage by which each node is supported was calculated with 200 bootstrap replicates, reporting the > 50% supported nodes.

Results

The first 4 PCs of GPA account for 95% of the total variance of APB (Table 2). MANOVA indicates that there is a significant variation in APB shape among species (Wilk’s Lambda = 0.005, F = 64.89, p < 0.05), and the post-hoc test confirms that each species is significantly different from the other (Hotelling’s p values < 0.05). The first two PCs, account for ~90% of the total shape variance and, as illustrated by the scatterplot in Fig. 2a, the individuals are clearly clustered in three groups, corresponding to different taxonomic units, according to these axes. The first axis (PC1) shows the separation between the new and the other two species, mainly due to the presence, in the former, of a large cone-shaped protrusion (horn) in the middle without angles or elevations at the ends of the ridge (see also Fig. 3). *P. orientalis* and *P. flavohirtus*, though clearly separated in two clusters, exhibit less variation: both species are characterized by the presence of angular-shaped lateral margins, but they differ in the median protrusion, rounded in *P. flavohirtus*, sharp in *P. orientalis* (see TPS grids along the Y-axis in Fig. 2a). The cluster analysis and the graphic representation via dendrogram, confirmed the relationship between the three taxonomic units and gave information about the grouping distribution. Two robust basal nodes divide the individuals of the three species (Fig. 2b). Individuals of the new species, collected from both sides of the Persian Gulf (Arabian peninsula and Iran) show a similar shape, clustering together. A robust node, confirmed by 100% bootstrap replicates, separates the new species from *P. orientalis* and *P. flavohirtus* complex. The latter two taxonomic units are divided by a further node (66%). As regards *P. flavohirtus* complex, individuals collected along the African coast of the Red Sea

Table 2 – Summary of the principal components (PCs) of the generalized Procrustes analysis (GPA) performed on the landmark configuration of anterior pronotal border, which described the 95% of the total shape variance.

<table>
<thead>
<tr>
<th>PCs</th>
<th>Eigenvalues</th>
<th>% Variance</th>
<th>Cumulative %</th>
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<tr>
<td>1</td>
<td>5.35E-03</td>
<td>79.97</td>
<td>79.97</td>
</tr>
<tr>
<td>2</td>
<td>5.02E-04</td>
<td>7.50</td>
<td>87.47</td>
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<tr>
<td>3</td>
<td>2.99E-04</td>
<td>4.48</td>
<td>91.95</td>
</tr>
<tr>
<td>4</td>
<td>2.14E-04</td>
<td>3.20</td>
<td>95.15</td>
</tr>
</tbody>
</table>

Fig. 1 – Scheme of the landmark configuration superimposed on the anterior pronotal border (APB) of *P. orientalis*: landmarks (white points and black numbers) on the horn apex and lateral ends of the APB ridge; semi-landmarks (black points and white numbers) set at regular intervals between landmarks.
Fig. 2 – Results of the Geometric Morphometric Analysis: a, scatterplot of the first and second PC resulting from the GPA with TPS transformation grids representing shape variation at the extreme of each axis; b, dendrogram of clusters resulting from UPGMA, with numbers indicating the percentage of bootstrap iterations (> 50%) that support each node.
New species of *Pseudoathyreus*

(Eritrea and Sudan) exhibit a similar shape and are separated from those collected in Western Africa (Senegal), though this node is not supported by more than 50% replicates. No clear geographical variation in shape pattern is highlighted for *P. orientalis*: all individuals examined show the same shape.

**Pseudoathyreus zianii** n. sp. (Fig. 4)

**Diagnosis**
The new species is easily distinguished from the other two species of the same group by the anterior border of pronotum with a wide-based and a more or less stout conic horn. On the contrary, *P. orientalis* and *P. flavohirtus* share a low transverse carina in the middle of APB with the profile of a narrow curly bracket, with either a small and pointed middle horn in the former species or a small and smoothed one in the latter. The central horn of the widespread African species *P. porcatus* (Laporte de Castelnau, 1840) looks a little like that of *P. zianii* but is larger, sharkfin-shaped, with a laterally compressed apex.

**Material examined**

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Fig. 3 – Anterior pronotal border (APB) of two specimens for each investigated taxonomic unit: a-b, *P. flavohirtus* complex from Fare (SA) and Massawa (ER); c-d, *P. zianii* n. sp. from Riyadh (SA) and Kerman (IR); e-f, *P. orientalis* from Jaisalmer (IN) and Pilani (IN). See Table 2 for country codes.

**Description of the Holotype**

Body length 15.20 mm, maximum width 9.10 mm. Bright brown colour. Anterior part of the body (head and pronotum) a little longer than elytra. Body dorsal surface scarcely setose, underside covered by dense setation.

**Head.** Labrum wider than long, without transverse carina, not emarginate in the anterior border. Mandibles asymmetrical, each with two protrusions on outer edge: posterior protrusions obtusely angulated and similar on both mandibles; anterior protrusion of right mandible well developed and distinct from the apex of the mandible which is hidden under the labrum; anterior protrusion of left mandible less developed and forming a rectangular angle with the apex which is well visible from above. Anterior transverse carina of clypeus, produced by a thickening of its anterior border, raised in the middle to form a small triangular backward figure (Fig. 4b). Posterior transverse carina (clypeo-frontal carina), delimiting the clypeus from vertex, forward leaning to cover partially the clypeal surface in dorsal view; slightly convex in dorsal view, it has the superior margin delimited by a tubercle on each end (Fig. 4b). An oblique clypeal carina, on both sides, connects clypeo-frontal tubercles to clypeo-genal angles. Fronto-vertex without swellings or other elevations. Genal angles rounded and raised upwards.

**Thorax.** Edges of pronotum entirely margined, except in presence of the lateral fovea on both sides, where they are deeply indented before the posterior angles (Fig. 4c). Base of pronotum, in the middle, obtusely pointed. Anterior margin of pronotum, in the middle, with a wide-based and stout conic horn, delimited on both sides by the anterior fovea and then by an elongated swelling which runs parallel to the antero-lateral margin of the pronotum itself (Fig. 4a). Two short and sinuate carinae, located on the highest point of pronotum (herafter: central carinae) delimitate a major excavation in its anterior half and a minor excavation towards the pronotal base (Fig. 4a). Two long swellings, curved inwards and accompanied by two immediately behind series of dense setae, start form the end of the central carinae and converge to the middle of pronotal base (Fig. 4a). A shallow but distinct median groove extends from anterior to posterior border of pronotum, through the bottom of its concavity. Pronotal sides are crossed by a convoluted carina that starts from a small anterior horn and turns over the lateral fovea. Fore coxa is visible through the indentation of the lateral fovea.

Left fore tibia with 5 teeth on outer edge, right fore tibia with 4 teeth (Fig. 4b). Ventral surface of fore tibia with indistinct punctuation and very fine rugosity. Scutellum small and narrow, rounded at apex, with very fine setae and punctures. Metasternum moderately convex, broadly rounded anteriorly, one third as wide as the body at that point; metasternal surface distinctly punctured and setose, crossed by a very fine, almost indistinct longitudinal groove near the posterior end. Middle coxae widely separated by metasternum, for a distance greater than width of labrum. Elytra shining and distinctly margined at base, with irregular rows of well-separated and fine punctures. Tarsus of fore and middle leg almost as long as tibia; tarsus of hind
leg longer than tibia. Hind tarsomeres elongated and slender, with dense piligerous punctures; first segment lacking a longitudinal carina. Longer hind tibial spur extending approximately to the half or the first third of the second segment.

**Abdomen.** Pygidium largely hidden by the elytra, with apex rounded. Ventrites very short compared to the length of the inferior body surface, and covered by a dense yellow pilosity. Aedeagus very small compared to body size, with short parameres curved downwards and inwards (Fig. 4d-e).

**Paratypes.** Body length 14.50 - 19.00 mm, width 7.50 - 13.00 mm. Color usually bright orange (lighter individuals) or bright brown (darker individuals), as expression of a chromatic sex-independent dimorphism. As in other species of Bobloceratinae, asymmetry in the number of teeth along the outer edge of fore tibia is frequent. In this species, in most cases, we found 5 teeth on left tibia and 4 teeth on right one. Sex dimorphism inconspicuous, limited to the slightly different shape of the last visible sternite (rounded in females; flat and transverse in males), also occurring in other genera of Bobloceratidae (see Carpaneto et al. 1990). Interspecific and intraspecific differences in male genitalia are not very marked in the genus *Pseudoathyreus*.

**Geographical distribution.** Eastern part of the Arabian peninsula (i.e. Kuwait, Saudi Arabian coast on the Persian Gulf, UAE, Oman), southern Iran, southern Pakistan.

**Name derivation.** Named for our colleague and friend Stefano Ziani, renowned specialist of Coleoptera Scarabaeidae, who sent us the material of his collection, including the first specimen that turned out to belong to the new species.

**Remarks.** Several individuals of this new species had been identified as *P. flavohirtus* (either in scientific literature or in museum collections), and appeared as such in both collections and pictures published in websites and social networks. This misinterpretation is due to the presence of individuals of *P. ziani* n. sp. determined by Boucomont in 1933 as “*Athyreus flavohirtus*” and as “*Athyreus porcatus* Cast. / v. flavohirtus Walk.” (both in the Natural History Museum of London). Also the totality of *Pseudoathyreus* occurring in the Tehran Museum (HMIM) were previously determined as *P. flavohirtus* by an anonymous identifier. Moreover, some of the specimens collected from Iran and UAE, identified as *P. orientalis* by Král & Batelka (2017) and Sommer et al. (2020), should be examined again because they could belong to *P. ziani* n. sp.

**Discussion**

As shown above, *Pseudoathyreus ziani* n. sp. clearly differs from the other taxonomic groups examined, because of the stout conical shape of the APB, without a basal supporting carina. Conversely *P. orientalis* and the *P. flavohirtus* complex differ among them for the middle horn shape, sharp (in the former) or smooth (in the latter). These diagnostic characters occur regardless of the body size and, as confirmed by GMA, are enough to highlight a clear clustering.

From a biogeographic point of view, these species, on the whole, occur throughout an horizontal arid corridor ranging from the Sahel dry savannas and semideserts of Africa to the driest areas of the Indo-Gangetic plain. They probably share similar ecological requirements, living in hot arid habitats mainly characterized by BWh climate type (B: arid, W: desert, h: hot arid), according to the climate classification of Kottek et al. (2006). Nevertheless, these species, as in general those belonging to the genus *Pseudoathyreus*, are usually allopatric because each locality usually harbours only one species, even though they can inhabit the same country, geographic area or administrative province. Their distribution range is clearly identifiable: the *P. flavohirtus* complex occurs from the arid savannas of northern Senegal to the Red Sea coast of Saudi Arabia; *P. ziani* occurs from easter Arabia to southern Pakistan (Ormara), with the northernmost location in Iran (Kerman); even if most of records of *P. orientalis* range between the Indus and Gange riverbasins, through the Thar desert up to western Bengal, we found specimens collected in northern Iran (Khorasan) and we also read literature records for Nepal and Tibet (Král &87u Batelka 2017; Sommer et al. 2020). Between Ormara, the south-easternmost location of *P. ziani*, and Karachi, the south-westernmost location of *P. orientalis*, there are no available data. More difficult is to define the northern border between these two species, because we did not see many specimens from that area. In any case, we could consider the Kirthar Mountains, between the two Pakistani provinces of Balochistan and Sindh, as the possible geographic border between *P. ziani* and *P. orientalis*. On the other hand, the Jabal Tuwaig escarpment and the Rub’ al Khali desert in central and southern Saudi Arabia could be a geographic barrier between *P. ziani* and *P. flavohirtus* complex.

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naturelle, Paris, France (Olivier Montreuil); NHM (=NHMUK), The Natural History Museum, London, UK (Maxwell V.L. Barclay); NHMB, Naturhistorisches Museum, Basel, Switzerland (Eva Sprecher-Uebersachs, Isabelle Zürcher-Pfander, Christoph Germann); NHMV, Naturhistorisches Museum, Vienna, Austria (Martin Lödl, Harald Schillhammer).

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