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A review of the genus *Eugaster* Serville, 1838 (Orthoptera, Tettigoniidae, Hetrodinae): a multifaceted approach

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Abstract

Overall coloration, size and thoracic morphology have formed the basis for taxonomic differentiation of taxa within the genus *Eugaster* at specific or subspecific levels over the years. The present study employs a range of methods to examine the morphology of 58 specimens (18♂♂ and 40♀♀) from Morocco, Algeria and Tunisia, collected from altitudes varying between 10 and 1795 metres AMSL. Moroccan sampling sites include localities on both the north and south of the High Atlas and from either side of the Middle Atlas, i.e., the Anti-Atlas, Western Meseta and High Plateau. The various techniques involve the characterisation of the five key colour forms and an investigation to examine links between colour form and geographical location and altitude, biometric analysis comprising selected variables, thoracic structure examination through feature extraction and edge detection, microscopy to examine male stridulatory files, an examination of the male genital sclerite structure for the presence of titillators, as well as molecular and phylogenetic analysis. Statistical tests are performed for results pertaining to biometrics, thoracic skeletisation, and the number of pegs on stridulatory organs. From results obtained, this study finds no basis to support the notion of the various taxa described in the past being assigned the rank of species and considers these to be infraspecific variants or forms. Consequently, the present authors propose to synonymise *Eugaster guyoni* (Serville, 1838) with *Eugaster spinulosa* (Johannson, 1763), resulting in the genus *Eugaster* being represented in North Africa by a unique but highly variable taxon, in terms of coloration, size and thoracic morphology.

Key words: Morocco, Algeria, Tunisia, morphometrics, DNA analysis, microscopy, stridulatory file, feature extraction

Introduction

The genus *Eugaster* Serville, 1838 is known from Morocco, Algeria and Tunisia (Serville, 1838; Chopard, 1943; Grzeschik, 1969), and is one of fourteen genera of the subfamily Hetrodinae Brunner von Wattenwyl, 1878, distributed across Africa, the Arabian Peninsula and parts of the Levant (Weidner, 1941; Schmidt, 1998; Grzywacz *et al*., 2015; Cigliano *et al*., 2023), within the Saharo-Arabian and Afrotropical zoogeographical realms (Holt *et al*., 2013). The geographical distribution of *Eugaster* encompasses steppic environments of varying degrees of aridity, from the Atlantic coast of Morocco, practically at sea-level, to locations of high altitude on the various Atlas Mountain ranges (e.g. Jebel Aberdouz >2,780m and Tizi n' Hamdoun 2,455m AMSL). Distribution extends eastward into Algeria and Tunisia, across the Tell and Saharan Atlas ranges, including the Aurès mountains, and adjoining plateaux, where rocky steppe abounds north of the Grand Erg Oriental. Although the genus is not characteristic of the Mediterranean biome, at least one taxon (*guyoni*) is known from a number of semi-arid locations that occur within the Basin's phytogeographical limits, such as the high elevations around Oran, Lalla Maghnia, Kef Oum Teboul (Algeria), Aïn Sfa, the Rif (Morocco), Gabès and Sfax (Tunisia), among others (Chopard, 1943; Sahnoun *et al*., 2010).

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Initially, the taxonomic rank ascribed to *Eugaster* was that of subgenus, with *Hetrodes* as nominal genus (Serville, 1838, pp. 463, 464, 770); the species *H. guyoni* Serville, 1838 was also described in the same publication (Serville, 1838, p. 464). Lucas (1861) separated *Hetrodes* and *Eugaster* on the basis of morphology, raising the taxonomic rank of the latter to that of genus. The proposed change appeared to have been recognised by a number of pioneering orthopterists (Finot, 1897; Bolívar, 1915; Caudell, 1916; Chopard, 1940, 1943), notwithstanding some persistence to retain subgeneric status (Stäl, 1874; Kirby, 1890). Kirby (1891; 1896; 1906) subsequently raised its taxonomic rank. The genus *Eugaster*, previously also encompassed other species known from east Africa, which Weidner (1955) separated on the basis of pronotal morphological differences. As a consequence, the genus *Eugasteroides* Weider, 1955 was created for some of the east African taxa (Weidner, 1955; Grzywacz, *et al*., 2015), while others, namely *Eugaster woodii* Kirby, 1891 and *E. suakimensis* Kirby, 1896 were placed in the genus *Anepisceptus* Fieber, 1853 in view of their long cerci (Schmidt, 1998); these were respectively synonymised with *A. revoili* (Lucas, 1884) [syn. *woodii*] and *A. horridus* (Burmeister, 1838) [syn. *suakimensis*] (Schmidt, 1998; Cigliano *et al*., 2023).

Chopard (1943) listed eight species, all described from Morocco with the exception of one taxon that was described from Algeria, notably, *Eugaster spinulosus* (Johansson, 1763) [TL: Maroc (?)], *E. guyoni* Serville, 1838 [TL: Algérie (♀)], *E. powysi* Kirby, 1891 [TL: Maroc (♂)], *E. fernandezi* Bolívar, 1935 [TL: (Morocco) Ifni, Sidi Ifni (♂, ♀)], *E. nigripes* Chopard, 1937 [TL: Maroc, Ifegh (♂, ♀)], *E. berlandi* Chopard, 1940 [TL: Maroc, Agadir (♂, ♀)], *E. laevigatus* Chopard, 1940 [TL: Maroc, Daiet er Roumi (♂)], and *E. mimeuri* Chopard, 1940 [TL: Maroc, Ifrane (♂, ♀)]. Of these, Chopard described no less than four taxa, in addition to *Eugaster spinulosus* var. *unicolor* Chopard, 1940 [TL: Maroc, entre Casablanca et Mazagan]. The dichotomous key provided by Chopard (1943) is based on coloration, shape of thoracic protuberances and denticles, and overall dimensions, which, in view of the broadly evident and significant variability among these taxa, has the potential for a fair measure of unreliability. Chopard's list (1943), published in his *Orthopteròides de l'Afrique du Nord*, interestingly mirrored those listed by Bleton (1942a), which the latter would have compiled from various sources, including presumably the original descriptions by Chopard. In the same year, Bleton (1942b) went on to describe two new species, namely *E. mathiasi* [TL: Tizitine (? Tizirine), 80 km SW of Meknès, Morocco] and *E. rungsi* [TL: Assoul, Morocco], which were subsequently synonymised respectively with *E. spinulosa spinulosa* (Johansson, 1763) and *E. guyoni nigripes* Chopard, 1937 (Grzeschik, 1969).

In the seminal contribution by Grzeschik (1969), the various taxa are grouped into two species, that is, *Eugaster spinulosa* (Johannson, 1763) and *Eugaster guyoni* (Serville, 1838), and further assigned subspecific ranking to the various forms or "variants", as Karl-Heinz Grzeschik prefers to refer to them (pers. comm. Grzeschik-Cassar/Massa, March 2023). A total of eight subspecies were assigned (Table 1), the nomenclature of which is still in current use (Grzeschik, 1969; Cigliano *et al*., 2023).

TABLE 1. The eight subspecies recognised by Grzeschik (1969) for *Eugaster spinulosa* and *Eugaster guyoni*.

E. spinulosa	*E. guyoni*
ssp. *spinulosa* (Johannson, 1763)	ssp. *guyoni* (Serville, 1838)
ssp. *powysi* Kirby, 1891	ssp. *fernandezi* Bolívar, 1935
ssp. *brevispina* Grzeschik, 1969	ssp. *nigripes* Chopard, 1937
	ssp. *berlandi* Chopard, 1940
	ssp. *intermedia* Grzeschik, 1969

In addition to differentiating between taxa on the basis of coloration and dimensions, Grzeschik's two-species split was essentially based on the notion of geographical separation of the two taxa by the High Atlas Mountain range, in effect, a physically impassable barrier, where the apterous, relatively heavy, and slow-moving *Eugaster* species are concerned (Grzywacz *et al*., 2015). To a fairly similar degree, the distribution proposed for *E. spinulosa* and *E. guyoni* in Morocco by Grzeschik (1969) draws interesting parallels that are not too different, albeit on a smaller geographical scale, from the distribution patterns of the tribe **Eugastrini** Karsch, 1887 (Cigliano *et al*., 2023), where the various genera are also largely separated by ecologically inhospitable terrain and/or impassable topography, such as desert or mountainous environments across hyper-arid regions of Africa and the Middle East. In terms of spatial patterns of species distribution, Grzeschik's case-study is effectively a microcosm of the broader **Eugastrini** distribution, wherein it is demonstrated how two closely related, possibly vicariant taxa are hemmed in

between inhospitable biomes and furthermore separated by the insurmountable elevations of the Moroccan High Atlas.

The overall topographic layout of Morocco tends to differ somewhat from that of Algeria and Tunisia. While the various ranges, including the Er-Rif, the Middle Atlas, the High Atlas and Anti-Atlas ranges encompass a significant portion of Morocco's territory, the Algerian Tell Atlas and the Saharan Atlas ranges tend to extend only across the country's northern segment, with the vast and extensive ergs (Great Western and Great Eastern formations north of the Tademaït Plateau) occupying territory all the way north to the southernmost foot of both the Ksour and Ouled Naïl mountains (Figure 1). In terms of ecological connectivity, it appears that the ergs (seas of sand on the Sahara platform) south of the Saharan Atlas would constitute a barrier to any southbound movement within Algeria and, likewise, eastbound movement by southern Moroccan *Eugaster* taxa. Contrarily, the High Plateau which extends from north-eastern Morocco into Algeria, between the Tell and Saharan Atlas ranges, is assumed to facilitate some form of connectivity to *Eugaster* taxa occurring north of the High Atlas. The same would apply in the case of Tunisia, in view of the physical alignment of the Aurès Mountains, which extend from eastern Algeria (Jolivet *et al*., 1999; Frizon de Lamotte *et al*., 2000, 2008; Michard *et al*., 2008).

FIGURE 1. The various mountain ranges north of the Sahara, across Morocco, Algeria and Tunisia (adapted from Doglioni *et al*., 1999). Such topographic elevations are assumed to have some influence on restriction of movement of *Eugaster* and potentially act as barriers to dispersal (Base map source: IUCN Map Server).

Separating the various taxa on the basis of coloration has possibly oversimplified, or even distorted, efforts for meaningful taxonomic determination. Conceivably, colour variation and varying sizes among *Eugaster* specimens may be the reason for the several described taxa over the centuries. As already alluded to above, in addition to Grzeschik's two-species split based on the notion of a physical topographic barrier (produced by the High Atlas Mts. range), he further characterised each of the two species in accordance with specific colour forms, namely 'whitish' (*E. spinulosa*) and 'brown or black' (*Eugaster guyoni*). It was proposed that these two principal colour forms, together with size and geographical distribution, were crucial morphological characters to distinguishing between the different subspecies (Grzeschik, 1969).

Defaut (1988), who encountered considerable difficulty in applying the taxonomic keys provided by Chopard (1943) and Grzeschik (1969) in view of the characteristically broad range of colour forms he came across within individual populations, made corresponding remarks. Having examined specimens from numerous localities within the Western Meseta, High Plateau, High, Middle and Anti-Atlas, Defaut (1988) suggested that any determination and/or separation of taxa should be based on stridulation and the structure of the male tittilators, rather than colour variation and geographical distribution, even if he concedes that these were not included in his analysis. In the same contribution, the author presented what he referred to as a provisional key (together with synonymised names) to determine three subspecies of *E. spinulosa*. These included ssp. *spinulosa* (Johansson, 1763) (syn. *laevigatus* Chopard, 1940) for the region north of the High Atlas and the High Plateau from low altitude to 1200 m AMSL;

ssp. *nigripes* Chopard, 1937 (syn. *mineuri* Chopard, 1940) for the Middle Atlas region, southward to the base of the High Atlas; and ssp. *fernandezi* Bolívar, 1935 (syns. *orientalis* Chopard, 1937; *berlandi* Chopard, 1940) for the region of the Anti-Atlas from the fringes of the Sahara northward to Souss. He further claimed not to have any familiarity with *E. guyoni* and proposed that the name *Eugaster powysi* be rejected since the species was described from a nymph and because the type locality given was vague (Defaut, 1988). The primary scope of this Introduction is not to present a critique of existing literature *per se*, but rather to outline key contributions on the subject matter; nevertheless, two fundamental issues emerge that merit elaboration. First, a name cannot be rejected or declared '*nomen dubium'* due to vague type locality or because the taxon was described from an immature stage (many old names, too numerous to mention, attest to this—see also the ICZN Code Articles 18 and 19). Second, there appears to be a lack of clarity, if not an element of confusion, pertaining to two of the names proposed as subspecies for *E. spinulosa*, namely, "*nigripes*", and "*fernandezi*", since these had already been assigned as subspecies to *E. guyoni* (Grzeschik, 1969), as indicated above, a species not familiar to the author.

Grzywacz *et al*. (2015) carried out a study in cytogenetics and molecular differentiation of African Hetrodinae, which included *Eugaster*. The Moroccan specimens used in the analysis were collected from demonstrably isolated populations on either side of the High Atlas range, from the following locations: El Kbab on the Middle Atlas; north of Marrakech on the Western Meseta; Amassine on the High Atlas; Tamaloukt on the southern flanks of the High Atlas; southeast of Taliouine on the foothills of the Anti-Atlas; and Tanalt and its north-eastern surroundings, and east of Tiznit on the Anti-Atlas mountains proper. Resulting phylogenetic data demonstrated low genetic differentiation among the samples examined, seemingly suggesting a single species. In view of the foregoing, Grzywacz *et al*. (2015) recommended a taxonomic revision of the genus *Eugaster*.

Heller *et al*. (2022) conducted a comprehensive study on the bioacoustics of **Hetrodini**, including *Eugaster*. Although this does not fall within the scope of the present study as such, the publication provides valuable data on stridulatory file morphology, as well as information about genitalic sclerites (titillator). Interestingly, the authors reported, in reference to titillators, that *Eugaster* and *Eugasteroides* do not appear to have "distinct sclerotized elements" (Heller *et al*., 2022, p. 454).

From the enthnobiological point of view, the taxa are known, vernacularly, as armoured ground crickets or "whistle crickets". It appears that some local herdsmen used the insect's body as a whistle for their livestock, after drying it and removing the limbs (University of Sydney museums webpage [last update: 04 Nov. 2010]: accessed 24 October 2023).

Aims—The present contribution sets out to investigate the taxonomic status of *Eugaster* taxa from localities across the Maghreb (in this case, Morocco, Algeria and Tunisia), through a range of approaches outlined in the Methodology below. In summary, this study seeks to examine *Eugaster* specimens (from across its entire north African range) from a diverse yet comprehensive set of perspectives, primarily focusing on (i) morphometrics, (ii) thoracic structure, (iii) stridulatory file and titillator (♂♂ specimens), and (iv) DNA analysis.

Methodology

As specified above, this contribution adopts a multifaceted approach to review and potentially revise the taxonomy of the genus *Eugaster*. Various techniques were employed, namely (i) biometric analysis; (ii) examination of the thoracic structure through feature extraction, involving background suppression and edge detection or 'skeletisation'; (iii) microscopy to examine the stridulatory file of male specimens; (iv) examination of the male genital sclerite structure with a view to ascertain whether or not titillators are present and, if so, sclerotized; and (v) DNA analysis. In addition, an assessment of colour variation (Table 3) in relation to sampling location was also made, with a view to investigate potential correlation between geographical provenance and colour forms.

A total of 58 specimens, comprising 54 adults (17♂♂ and 37♀♀) and four nymphs (1♂ and 3♀♀) from twenty locations (Table 2), were available for the present study. Of these, 53 adult specimens were used for biometric analysis and for the initial part of the thoracic structure 'proportional morphometric analysis'; 24 (10♂♂ and 14♀♀), selected on the basis of colour form variation and geographical location, were subsequently used for the thoracic structure comparative assessment. Twelve male specimens (selected from localities north and south of the High Atlas) had their tegmina extracted to expose the stridulatory file; six males, again from either side of the High Atlas, were examined for the presence of titillators; and 15 adults (2♂♂ and 13♀♀), representing different colour forms

from as broad a geographical representation as possible, underwent molecular analysis. Location data and colour form distribution of all specimens is illustrated in Figure 4 (A and B), while a more holistic suite of data, inclusive of morphometric measurements, is listed in Table 12.

Abbreviations for collections and museums:

ACPC: Aldo Catania, personal collection, Haz-Zebbug, Malta
BMPC: Bruno Massa, personal collection, Palermo, Italy
LFCPC: Louis-F Cassar, personal collection, San Pawl tat-Targa, Naxxar, Malta
MSNG: Museo Civico di Storia Naturale 'G. Doria', Genoa, Italy
NMNH: National Museum of Natural History (Heritage Malta), Mdina, Malta

TABLE 2. List of localities from where specimens were collected (or in proximity of) and respective codes.

Code	Locality	Code	Locality
EM-Mrk	(Eugaster) Morocco, Marrakech	EA-eKn	(Eugaster) Algeria, El Kantara
EM-Mid	(Eugaster) Morocco, Midelt	EA-BSa	(Eugaster) Algeria, Bou Saada
EM-Set	(Eugaster) Morocco, Settat		
EM-Tal	(Eugaster) Morocco, Taliouine (two sites)	ET-Tmz	(Eugaster) Tunisia, Tamerza
EM-Alz	(Eugaster) Morocco, Aoulouz	ET-JCb	(Eugaster) Tunisia, Jebel Chambi
EM-GdT	(Eugaster) Morocco, Gorges du Todrha		
EM- Orz	(Eugaster) Morocco, Ouarzazate		
EM-Anz	(Eugaster) Morocco, Anezal		
EM-Tzt	(Eugaster) Morocco, Tiznit		
EM-Azu	(Eugaster) Morocco, Azrou		
EM-Rbt	(Eugaster) Morocco, Rabat		
EM-SIf	(Eugaster) Morocco, Sidi Ifni		
EM-Taz	(Eugaster) Morocco, Taznakht		
EM-SBO	(Eugaster) Morocco, Sidi Bou-Othmane		
EM-AaA	(Eugaster) Morocco, Aarba ait Ahmed		

The main colour forms of *Eugaster* taxa, as characterised by the present study, are the following: black (at times with blood-red thoracic spines and posterior pronotal region); uniformly brown (with individuals varying from a dark to a sandy hue); particoloured with ochre and a range of light to medium brown tones with black markings; and, an overall cream colour with black and/or dark brown markings with ochre patches. Notably, individuals from Sidi Ifni (the type locality of subspecies *fernandezi* Bolívar, 1935) appear to be predominantly tricoloured, comprising an auburn pronotum, light brown head and limbs, and a dark brown-auburn chequered abdomen (refer to Table 3 for details and Figure 2 for comparative purposes). Together with these, it transpires that some intermediate colour variants also occur.

Photographs of adult specimens were taken with a Canon EOS 7D Mark II and a Canon EF 100 mm f/2.8 macro lens set at f/8 and adjusted to manual exposure mode with ring flash mounted on a tripod, and with a Nikon Coolpix 995 mounted on a Wild Heerbrugg M3 stereomicroscope. The high-resolution images produced were used for colour form assessment and supplementary morphological evaluation, as also for the investigative work related to feature extraction and edge detection with a view to thoroughly examine the thoracic structure (including the spines or protuberances).

TABLE 3. Characterisation of the main *Eugaster* colour forms (see also Figure 2).

Colour forms	Explanatory notes
Black, overall, with red or black thoracic spines and posterior pronotal region. (*Code*: **Blk O**)	Some individuals display some medium brown coloration on the scutellum, scutum and abdominal tergites [T1, T2]; on the tibiae; and/or the pronotum. (Figure 2 **A–C**).
Brown with black markings on all three main body parts, banding on limbs, and a chequered abdominal pattern. (*Code*: **Br/bm**)	Ranging between light/medium browns to ochre (noticeable variability within and among individuals in a population). Thoracic tubercles and spines black; posterior pronotal region mostly ochre (Figure 2 **D–F**).
Cream with black markings on all three main body parts, banding on limbs, and a chequered abdominal pattern. (*Code*: **Cr/bm**)	Thoracic tubercles, spines, and posterior pronotal region black (Figure 2 **G**); sparse patches of ochre are sometimes present on the pronotum, abdomen and limbs of some individuals. (This may also be considered a variant of the preceding form).
Uniformly Brown. (*Code*: **U Brn**)	Broad variability in the shades of brown, ranging from sandy to dark brown (Figure 2 **H**); with darker abdominal coloration, including banding, in some individuals.
Trichromatic (auburn, light/dark brown). (*Code*: **Trkrm**)	Pronotum (inclusive of thoracic tubercles and spines) uniformly auburn (Figure 2 **I**); light brown head and limbs (latter with dark flecks), with patches of such coloration on the scutellum; and a dark brown abdomen with auburn coloration forming a suffused chequered pattern.

Various statistical tests were performed. ANOVA (analysis of variance) was employed for biometric assessment of adult specimens, to compare the means of the different colour forms and to determine whether the means of the considered parameters are significantly different. This was carried out separately for all adult male and female individuals.

A Pearson correlation analysis was carried out to test for potential correlations among dimension parameters of the thoracic structure; this was carried out separately for all the adult male and female specimens available for this study. The test focused on various variables, namely (i) length from the upper rim of the pronotum to the tip or knee of the hind femur, (ii) length of the pronotum, (iii) width of the thorax inclusive of spines, and (iv) width of the thorax excluding the spines.

In the case of the stridulatory file assessment, attention was focused on length of the entire organ and the number of pegs on the file of the left tegmen, even though both the left and right tegmina of selected male specimens were examined. Specimens belonged to the two most common colour forms (**Blk O** and **Br/bm**), collected from two representative geographical regions, that is, the Western Meseta and the Anti-Atlas, located on either side of the High Atlas. Details of the respective statistical tests are included in the results below.

Biometric assessment and statistical analysis—53 adult specimens were subjected to a biometric assessment, using an electronic digital calliper (resolution: 0.01mm/accuracy: ±0.02). Measurements of firmly sclerotized parts of the body structure, namely the pronotum and femur, were carried out (the abdomen was not included in view of the presence of less strongly sclerotized membranes). The following specific measurements were taken: (i) length of pronotum; (ii) length from the upper rim of the pronotum to the outer tip of the femur; (iii) thorax width with spines (at the widest point of the protuberances); and (iv) thorax width (excluding protuberances).

Feature extraction and edge detection—An analysis of the thoracic structure was carried out on 53 adult specimens, with a view to determine whether each individual's thoracic dimensions (length and width) were proportional to its overall size. Subsequently, 10♂♂ and 14♀♀ from different geographical regions and representing all five colour forms were selected for a comparative assessment of the thoracic structure. All specimens were photographed with a tripod-mounted DSLR camera (specs. outlined above) that was positioned equidistantly from the plastazote-lined examination tray on which each of the specimens was placed. Each resulting image underwent an initial feature selection process to 'isolate' the specimen, which was then exported as a high-resolution TIFF file. Subsequently, all photographs were imported into Adobe Photoshop, where the white background was meticulously removed in order to display each specimen against a transparent backdrop. While automated tools were utilised

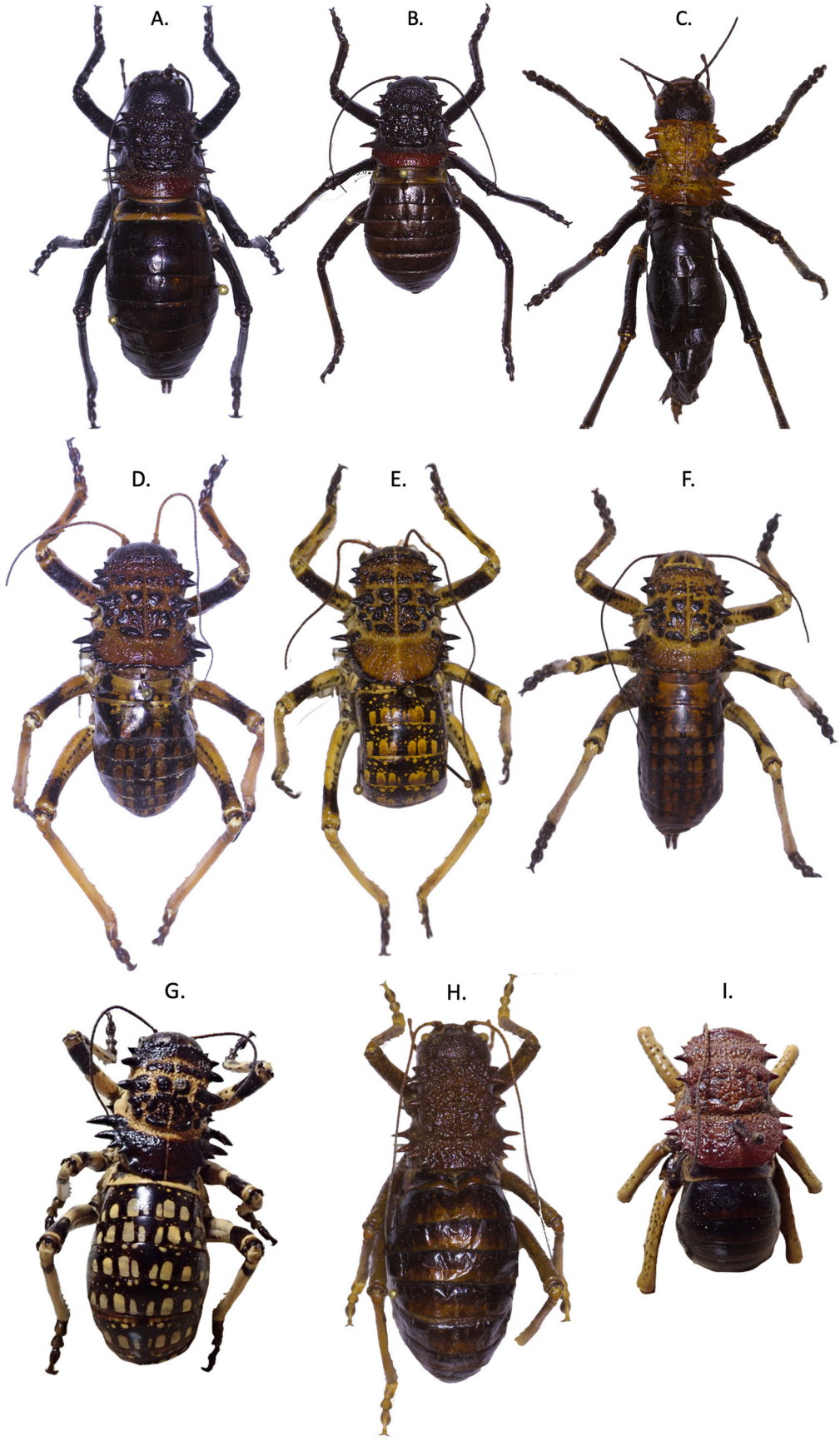

FIGURE 2. Main *Eugaster* colour forms, as characterised in Table 3. **A**–**C**: Blk O; **D**–**F**: Br/bm; **G**: Cr/bm; **H**: U Brn**; I**: Trkrm. ***Note:*** C [specimen code: EM-Mid-4], from the High Plateau, is considered, to some extent, atypical.

during the process of *skeletisation*, manual corrective tracing of each individual specimen's outline was also deemed necessary (i) to ensure preservation of selected, intricate morphological characters, and (ii) to eliminate from the image those body parts (e.g., abdomen and limbs) deemed inessential to this component of the analysis. After a thorough quality check of each individual outcome to ensure that key attributes had not been inadvertently omitted or distorted, the images were converted to grayscale, and an edge detection algorithm was applied to extract each specimen's outline (Figure 3).

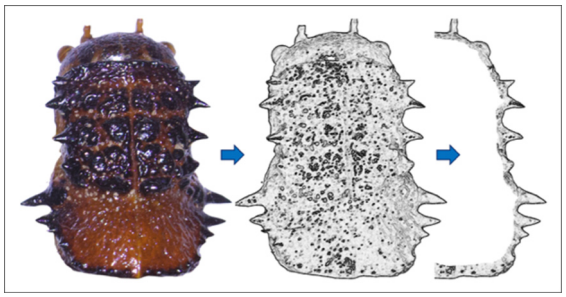

FIGURE 3. Images of the 3-phase process involving feature extraction and edge detection to attain the *skeletised* outline.

Examination of stridulatory file—12♂♂ specimens from localities on either side of the High Atlas range had their tegmina extracted to expose the stridulatory file for microscopic investigation. Two methods were employed to rehydrate the selected male specimens, namely (i) partial immersion for <1 hour in tepid water, to which a few drops of antiseptic were added to prevent any subsequent development of mould; and (ii) via the use of a 'relaxing chamber' in which specimens were placed on a plastazote platform (with a view to minimize direct contact with the rehydrating fluid beneath) and left for approximately 24 hours, during which time the rehydration process rendered the specimens malleable enough to handle without any risk of potential damage. Once extracted, each pair was attached to a mounting card using water-soluble glue. Each specimen's tegmina and stridulatory organ were photographed for subsequent examination (number of pegs or teeth, and length), using a Leica DVM6 digital microscope with a FOV of 43.73 and Z-stacking capability.

Genital sclerite structure examination—Six male specimens, ensuring representation from either side of the High Atlas Mountains, were selected for investigation of the presence of genitalic sclerites. Such anatomical characters, in particular titillators, are considered key taxonomic characters in determining various groups of Tettigoniidae; the dissection of terminalia traditionally employs the use of a KOH solution to foster maceration of tissue around sclerotized copulatory structures, after which the chitinous organ would be mounted for examination under the microscope. However, since it was recently reported by Heller *et al*. (2022) that the titillators of *Eugaster* taxa may not be sclerotized, two approaches were adopted, the first based on the traditional KOH solution (applied to two specimens), and the second using (i) warm water with drops of liquid antiseptic (applied to two specimens) and (ii) a commercially available 'relaxing fluid' prepared by a specialist supplier (applied to two specimens). A trinocular stereo zoom microscope (8×–50×), fitted with a 12 MP digital camera, was also used during the latter part of the investigative procedure.

Molecular methods—DNA was extracted from middle or hind femora of fifteen dry specimens using the QIAamp DNA tissue microkit (QIAGEN) and quantified using a Nanodrop 2000 spectrophotometer. For the sake of standardisation, the primer pairs used were similar to those used in Grzywacz *et al.* (2015), Chintauan-Marquier *et al.* (2016) and Geller *et al*. (2013). These included DNA markers as used in previous phylogenetic studies of crickets (Chintauan-Marquier *et al*., 2016) as well as in the only genetic study, prior to the present effort, of *Eugaster* (Grzywacz *et al*., 2015). These included the mitochondrial gene coding for cytochrome b protein (cytb, ~400 bp), the cytochrome c oxidase I (COI), the large (16srRNA, ~500 bp) and the small (12SrRNA, ~400 bp) mitochondrial ribosomal subunits, a fragment of the small nuclear ribosomal subunit (18SrRNA, ~650 bp), two fragments of the large nuclear ribosomal unit (28SA, ~400 bp and 28SD, ~900 bp rRNA), the gene coding for H3 protein (H3, ~330 bp), 18S rDNA (18S, ~818 bp), fragment of ITS1 (ITS1, ~444 bp), and fragment of ITS2 (ITS1, ~409 bp). PCR amplifications were performed in a total volume of 25 µL, containing approximately 12.5 ng of DNA, 12.5 µl of OneTaq QL 2X MM Standard Buffer (NE Biolabs), 1 µl of 10 µM of each primer, and 10 µL nuclease-free water. These were carried out in a GeneAmp thermocycler PCR system 2700 (Applied Biosystems,

Foster City, CA, USA) or T3000 thermocycler (Biometra, Jena, Germany) according to the PCR protocols listed in aforementioned literature (Grzywacz *et al.,* 2015;Chintauan-Marquier *et al.,* 2016), as well as the following PCR conditions for the COI sequences: 95 °C for 5 min followed by 35 cycles of 95 °C-30 sec, 48 °C-40 sec and 72 °C-50 sec and elongation 72 °C-7 min. PCR products were verified on 1% (w/v) agarose gel. Some of the molecular work was performed at the Biome-id in Wilhelmshaven, Germany.

Phylogenetic analyses—The sequences were manually checked by inspecting the chromatograms and subsequently compared to published sequences using the Basic Local Alignment Search Tool (BLAST) of the United States National Centre of Biotechnology Information (NCBI) (Zhang *et al.,* 2000). The nucleotide sequences obtained in this study were deposited on the GenBank and assigned an accession number. Alignments of the COI biomarker were performed using the MAFFT algorithm L-INS-I (Katoh & Standley, 2013) on the NGPhylogeny portal (Lemoine *et al*., 2019). A dataset, based on COI sequences (661 nt), was analysed for species belonging to the Tettigoniidae as per Grzywacz *et al.* (2015). This included eight nucleotide sequences from GenBank, together with two new sequences produced in this study. *Saga hellenica* Kaltenbach, 1967 was used as outgroup. Maximum Likelihood (ML) analyses was carried out using PhyML (Guindon *et al.,* 2010) on the NGPhylogeny portal (Lemoine *et al*., 2019), with the general time reversible + gamma distribution + invariable sites model (GTR + G + I) (Nei & Kumar, 2000). This was determined from the Maximum Likelihood scores implemented in jModelTest 2.1 software (Darriba *et al*., 2012), with 1000 bootstrap replicates. Bayesian Inference (BI) was performed using MrBayes v. 3.2.7 (Ronquist *et al*., 2012) on the NGPhylogeny portal (Lemoine *et al*., 2019). BI analyses were run with the GTR + G + I model parameters estimated independently for each partition, with four Monte Carlo Markov Chains for 2 million generations. Nodal support was assessed by calculating the posterior probability (PP) values for each node of the resulting consensus tree after a burn-in value of 25% of the trees. Both ML and BI analyses produced trees with a similar topology.

Results

The findings of this multifaceted research are presented in sections and subsequently addressed holistically for integration of results.

Colour forms—The resulting spatial distribution maps of the main colour forms (Figure 5 A and B) demonstrated three key outcomes. First, a well-defined clustering of colour forms, specific to location, is especially noticeable, with no evidence of any two main colour forms occurring within the same locality. Such finding does not only result from the suite of specimens examined for the present contribution, but also from direct field observations carried out independently by two of the authors [BM and LFC], as also by other colleagues (pers. comm. J. J. Borg/A. Catania/A. Seguna-Cassar, 2023) during their field study visit to Morocco in 2006. Second, the two most pervasive colour forms, namely the overall black form often with some blood red coloration on the pronotum (code: Blk O), and the light-coloured form with chequered markings on the abdomen (code: Br/bm), occurred on either flank of the High Atlas, with the former also occurring on either side of the Middle Atlas, that is, on the Western Meseta (*loc.* Settat) and the High Plateau (*loc.* Azrou), and as far east as El Kantara in Algeria. Specimens of the latter (Br/bm), which were examined for the present study, were recorded from the Western Meseta (*loc*. Sidi Bou-Othmane and north of Marrakech) and from the Anti-Atlas (*loc*. north of Tiznit), as well as from around Jebel Chaambi in Tunisia (4 and 5). On the basis of records pertaining to this study, other main colour forms were noticeably more spatially restricted. For example, the lighter, cream-coloured variant with chequered markings (code: CR/bm) was recorded only on the Western Meseta, the tri-coloured form (paratype of *E. fernandezi*) solely at Sidi Ifni on the coastal foothills of the Anti-Atlas, and the uniformly brown form (code: U Brm) from hyper-arid locations of Arbaa ait Ahmed on the Moroccan Anti-Atlas and Tamerza in Tunisia (Figures 5 A and 5 B).

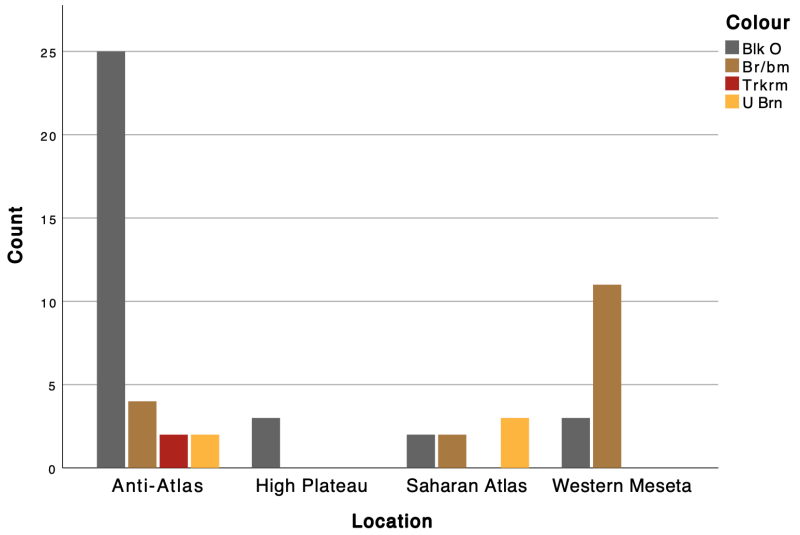

FIGURE 4. Regional distribution of colour forms of specimens examined for this study.

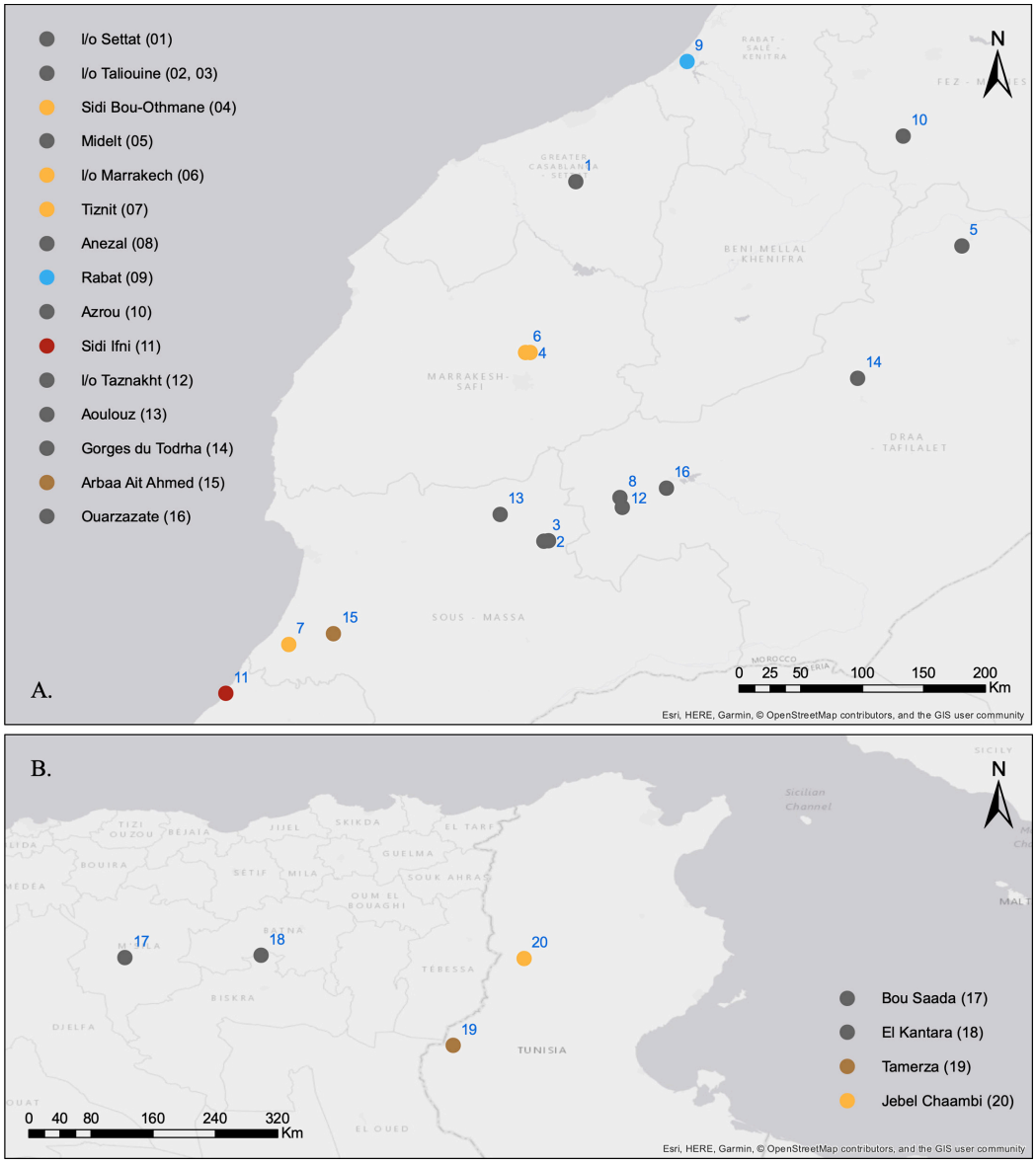

FIGURE 5 A [*top*] and **B** [*bottom*]. Spatial distribution of all records pertaining to the present study, for sites in Morocco [A] and sites in Algeria and Tunisia [B], based on specimen data. ●Blk O; ●Br/bm; ●Cr/bm; ●U Brn; ●Trkrm.

Apart from these generally recognised colour forms, additional marked variances in coloration, albeit minor, do occur. For instance, a number of specimens belonging to the overall black form (Blk O) were recorded from Midelt, on the High Plateau, at the end of June 2006. Their coloration was either characteristically black with blood red thoracic spines and posterior pronotal region, and with a vivid ochre background on the entire pronotum, or entirely black. Although both variants of the overall black form seemed abundant at the time of observation, the majority of individuals encountered belonged to the former. Most were observed, in significantly large number, on a stony plain environment or reg-type terrain (Figure 6 A), as also along the verge of the thoroughfare between Midelt and Aouli (Figure 6 B) and on the road proper, where roadkill incidence was conspicuously high. Several were being consumed by individuals while seeking to cross the asphalted road (pers. comm. J.J. Borg-Cassar, 2023).

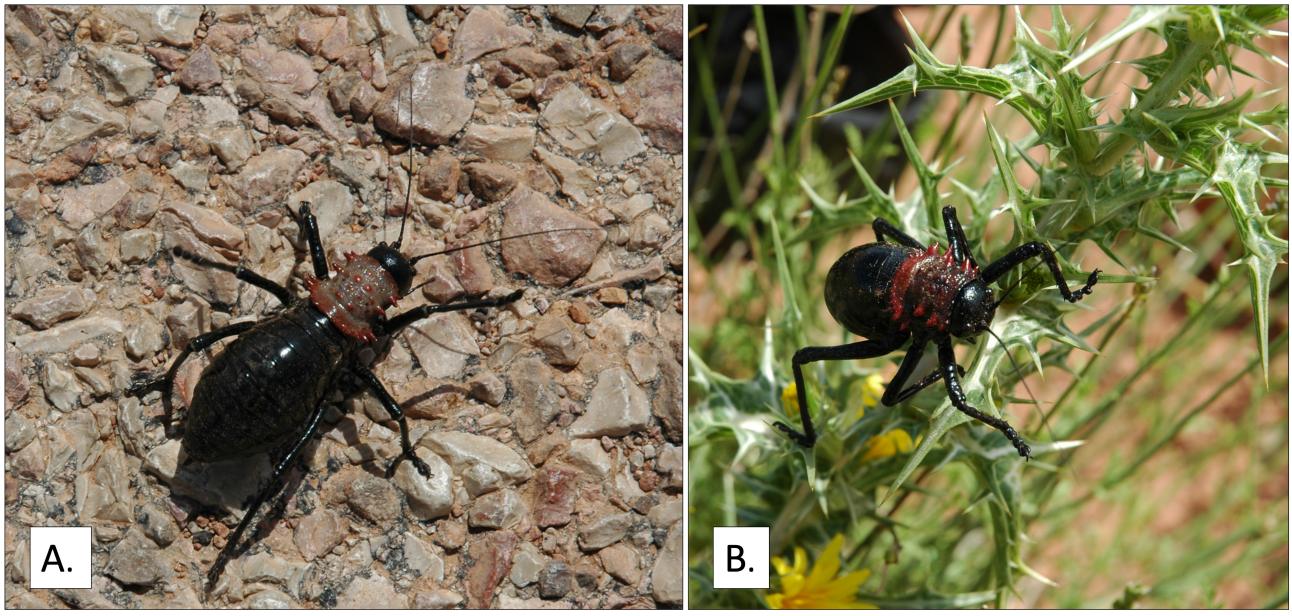

FIGURE 6 A [*left*] and **B** [*right*]. Female specimens in the vicinity of Midelt (High Plateau) in June (Photos: P.M. Sammut).

The third and perhaps most significant finding, where colour forms are concerned, is the fact that the black colour form (Table 3, colour code **Blk O**) occurred at altitudes of between 700 and 1795 metres above mean sea level, with one exception, that of a single specimen taken at Settat, south of Casablanca, on the Western Meseta [specimen code: EM-Set-9]. If the location data is indeed correct, then this would be the only anomaly for this colour form in terms of altitude. The altitude of other sites within which this colour form was recorded in Morocco always exceeded 1200 m AMSL [Azrou on the Western Meseta—Middle Atlas boundary at >1200 m; Aoulouz at ≈1250 m; two localities in the limits of Taliouine at 1425 m and 1483 m respectively; Ouarzazate at 1450 m; 20 km north of Taznakht at 1510 m; and, Anezal all on the Anti-Atlas at 1550 m; Midelt at 1677 m on the High Plateau; and, Gorges du Todrha on the High Atlas—Anti-Atlas interface at 1795 m]. In Algeria, the **Blk O** colour form occurred at a marginally lower altitude, namely at ≈700 m at Bou Saada and 710 m on Jebel Metlili at El Kantara.

The brown colour form with black markings (Table 3, colour code **Br/bm**; Figure 7) was recorded at altitudes that ranged between 210 and 560 metres AMSL [North Tiznit at 210 m on the Anti-Atlas; Sidi Bou-Othmane at 516 m; and, north of Marrakech at between 516 m and 560 m, all three sites on the Western Meseta; and, on Jebel Chaambi, within the Aurès range, in Tunisia at 490 m]. The specimens pertaining to the cream colour form with black markings (Table 3, colour code **Cr/bm**) were recorded near Rabat, on the Western Meseta at an altitude of <95 m AMSL.

The uniformly brown colour form (Table 3, colour code **U Brn**) was recorded at practically the two extreme ends on an East–West axis of the *Eugaster* distribution range, that is, at Arbaa ait Ahmed on the Anti-Atlas, near the Atlantic coast of Morocco, and Tamerza (Tunisia), located on the eastern extreme of the Aurès mountain range (which extends from eastern Algeria to western Tunisia), respectively, at altitudes of 360 m and 10 m AMSL.

The trichromatic colour form (Table 3, colour code **Trkrm**), with a relatively unique coloration, was recorded solely from the coastal area at Sidi Ifni on the Anti-Atlas, at an altitude of <15 m AMSL.

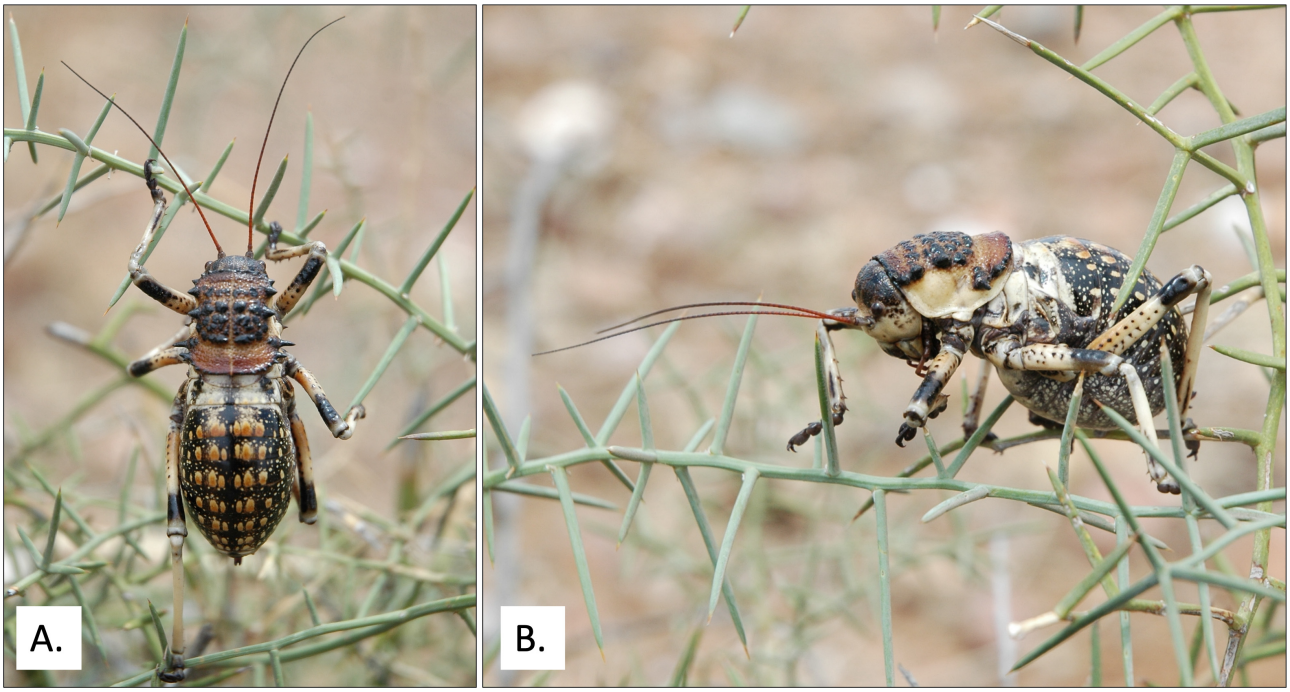

FIGURE 7 A [*left*] and **B** [*right*]. Female specimens at Sidi Bou-Othmane (Western Meseta) in June (Photos: J.J. Borg).

Whether or not the various colour forms belong to different species, it is assumed that some parallel exists between colour form variation and surrounding landscape, extant biotope, and/or prevailing environmental conditions. Might the physiological significance of the dominant black coloration of individuals occurring within high altitude regions be a thermoregulatory adaptation, in line with the *thermal melanism hypothesis*? (Huey & Kingsolver, 1989; 1993; Clusella-Trullas *et al*., 2007, 2008). Conversely, might the lighter coloured individuals that occur at lower altitude be adapted to enhanced reflective capability to prevent excessive heating of the body? Such physiological adaptation may be analogous with that of the west African cetoniid, *Goliathus goliatus* (Drury, 1770), for which it has been suggested that coloration of elytra (which range from white to dark brown) may be contingent upon the forest type (open or closed canopy) in which the larvae develop, as a result of the amount of light that actually penetrated to the forest floor (Sibilia *et al*., 2018; Zverev *et al*., 2018; Dendi *et al*., 2021). Black *Eugaster* forms, as would be the case with *Goliathus*, would conform with the hypothesis, since these large, dark-coloured exotherms will tend to benefit in cooler climatic conditions of high-altitude locations, since it is envisaged they would absorb more solar radiation than their lighter counterparts. Colour form distribution in relation to altitude is presented in Figure 8. As indicated above, apart from a single exception of a **Blk O** individual taken at Settat (altitude: 252 MASL), all other records demonstrate a clear correlation between colour form type and altitude.

Biometric assessment and statistical analysis—Visually, some individual specimens appear to be different from others, not only in terms of coloration, but also in respect of some physical attributes. ANOVA was performed, separately for males and females, to compare the means of all colour form types, as well as to determine if the means of selected biometric parameters [length of pronotum; length from upper rim of pronotum to femoral tip (knee); thorax width with spines; thorax width excluding spines] are significantly different (full data in Table 12).

In all cases pertaining to male specimens, the *p*-value is greater than .05 and there is therefore **no statistically significant difference** between the means of the parameters considered among the five colour form types (Table 4).

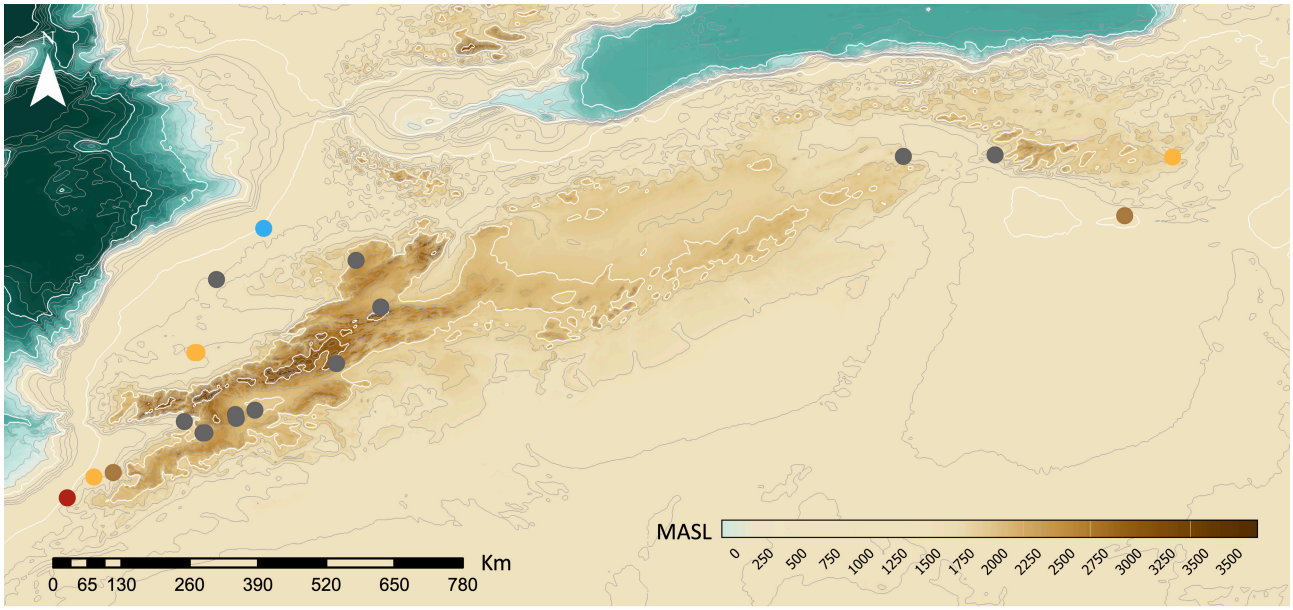

FIGURE 8. Contour map highlighting altitude—colour form gradient across localities in which specimens were recorded (Base map source: ETOPO).

TABLE 4. ANOVA test output for the male specimens measured in this study.

		Sum of Squares	df	Mean Square	F	Sig.
Length Pronotum	Between Groups	8.190	3	2.730	.687	.576
	Within Groups	51.683	13	3.976		
	Total	59.872	16			
Length Pronotum – Femur Tip (Knee)	Between Groups	75.366	3	25.122	1.705	.215
	Within Groups	191.520	13	14.732		
	Total	266.886	16			
Thorax width + Spines	Between Groups	10.674	3	3.558	1.729	.210
	Within Groups	26.758	13	2.058		
	Total	37.433	16			
Thorax width excl. Spines	Between Groups	2.438	3	.813	1.393	.289
	Within Groups	7.584	13	.583		
	Total	10.022	16			

In the case of the female specimens, the mean of the parameters relating to biometrics of the pronotum [length of pronotum; length from upper rim of pronotum to femoral tip (knee)] were **not found to be significantly different** between the different colour form types, with *p*-values again above the .05 threshold, as in the case of the males. However, differences in the means of parameters involving biometrics of the thorax [thorax width with spines; thorax width excluding spines] were **significantly different** between the colour form types (Table 5). The spines or thoracic protuberances are further discussed in the next sub-section (Feature extraction and edge detection).

TABLE 5. ANOVA test output for female specimens measured in this study.

		Sum of Squares	df	Mean Square	F	Sig.
Length Pronotum	Between Groups	29.414	4	7.353	1.561	.209
	Within Groups	146.039	31	4.711		
	Total	175.453	35			
Length Pronotum – Femur Tip (Knee)	Between Groups	167.426	4	41.857	1.990	.121
	Within Groups	652.046	31	21.034		
	Total	819.472	35			
Thorax width + Spines	Between Groups	43.959	4	10.990	3.198	.026
	Within Groups	106.545	31	3.437		
	Total	150.504	35			
Thorax width excl. Spines	Between Groups	30.302	4	7.575	6.211	<.001
	Within Groups	37.807	31	1.220		
	Total	68.109	35			

Feature extraction and edge detection—A cursory glance at the thoracic structure of the various colour forms gives the impression of characteristically very diverse outlines; the examples in Figure 9 are a case in point. Yet, when one looks carefully at the number, configuration and sequence of protrusion of each thoracic spine, it soon becomes evident that there is minimal distinction between these, even if some protuberances may be different in terms of shape, with some edges being rounded and often stocky, while others thin and more pointed.

Pearson's R (Pearson correlation coefficient) tests analysed the relationship between selected variables pertaining to or associated with the thoracic structure (length from upper pronotum tip to the knee of hind femur, pronotum length, thorax width including spines, and thorax width excluding spines) (Table 6). Scatter graphs were generated for each pair of variables. In the case of male specimens, a strong correlation was observed between the length of the pronotum-hind femur tip and thorax width (both including and excluding spines), with significance at the 0.01 level. In the case of female individuals, all parameters exhibited significant correlations at the 0.01 level.

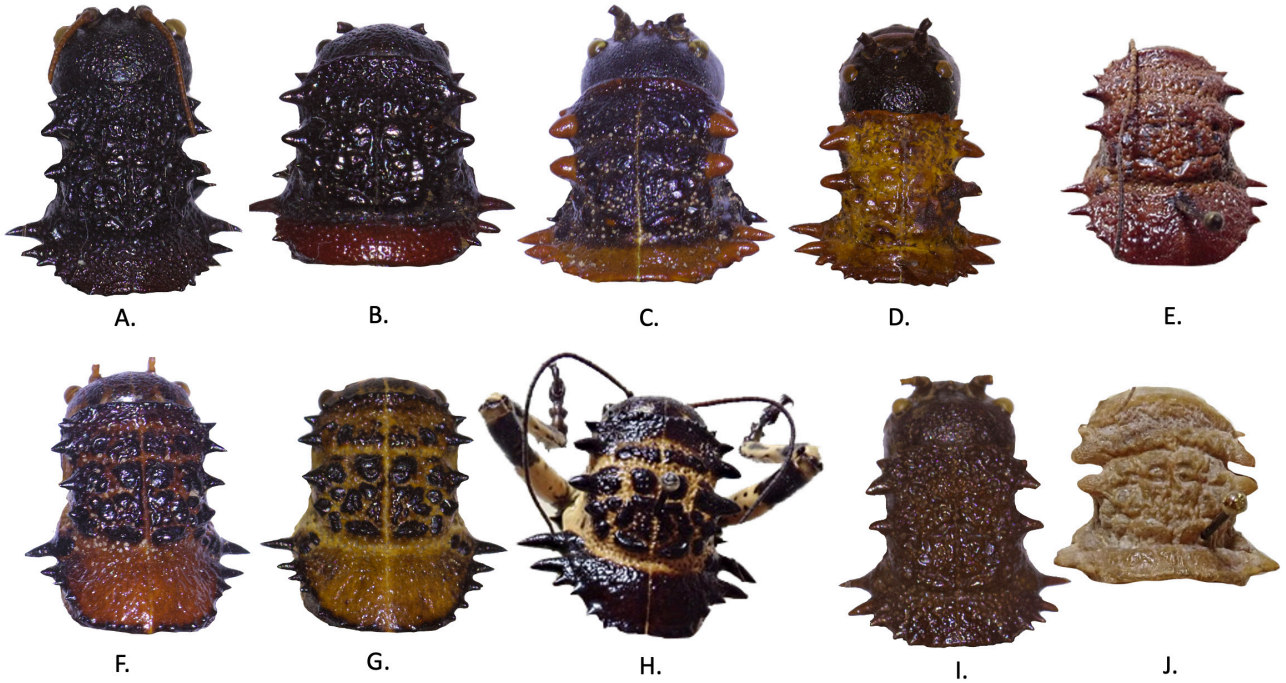

FIGURE 9. Head and thoracic regions of the various colour forms and variants, which marked the initial phase of the *feature extraction and edge detection* process. **A**–**D**: Blk O; **E**: Trkrm; **F**–**G**: Br/bm; **H**: Cr/bm; **I**–**J**: U Brn.

Visualisations indicated that all samples followed a general linear trend, with only one male sample (specimen code: EM-SBO-6 ♂) being identified as a clear outlier. There was also one outlier among the female cohort, which however was not as pronounced as its male counterpart. The specimen (code: ET-Tmz-53 ♀), which appears to exhibit some manner of deformity (Figure 9 J) in terms of thoracic morphology, can be considered appreciably uncharacteristic. Its inclusion in Figure 9 was purely to illustrate the 'uniform brown' (U Brn) colour form. From the demonstrable strong linear relationship (particularly evident for the male specimens) resulting from the Pearson Correlation Coefficient tests, it can therefore be concluded that the size (length and width) of the thoracic structure of each individual insect is generally proportional to its overall size.

TABLE 6 A [*top*—males] and **B** [*bottom*—females]. Correlation matrix for variables associated with the thoracic structure of male and female specimens, respectively, summarising the bivariate Pearson correlation values for all the variables evaluated. Correlation is significant at the 0.01 level (2-tailed). Values in bold within shaded cells show statistically significant correlations.

A. Males	Length pronotum—Hind femur tip	Pronotum length	Thorax width + spines	Thorax width excluding spines
Length pronotum—Hind femur tip	1	0.248	**0.674**	**0.673**
Pronotum length	0.248	1	0.45	0.354
Thorax width + spines	**0.674**	0.45	1	0.322
Thorax width (excluding spines)	**0.673**	0.354	0.322	1

B. Females	Length pronotum—Hind femur tip	Pronotum length	Thorax width + spines	Thorax width excluding spines
Length pronotum—Hind femur tip	1	**0.716**	**0.852**	**0.607**
Pronotum length	**0.716**	1	**0.814**	**0.746**
Thorax width + spines	**0.852**	**0.814**	1	**0.821**
Thorax width (excluding spines)	**0.607**	**0.746**	**0.821**	1

Although *Eugaster* thoracic cuticular armour morphology had often been suggested as a means of characterisation to determine taxa at species or subspecies level by various scientific workers, the statistical analysis carried out, results of which are presented in Tables 6 A and 6 B, and Figures 10 A and 10 B, demonstrate that the entire suite of specimens examined is biometrically analogous. This observation is further validated by results obtained from the edge detection process (Figures 11 A and 11 B).

Examination of stridulatory file—Following extraction of the tegmina, high resolution images of the sclerotized forewings and stridulatory files were taken, first in pairs (Figure 12 *top* and *middle*) and then singly (Figure 12 *bottom*). The reason for the former was to compare the stridulatory file on the left tegmen with that on the right tegmen within each pair, while the latter allowed a closer and more detailed examination of the pegs (also referred to as serrations or teeth) on individual sound-producing structures. In addition to functioning as stridulatory mechanisms, these also function as proprioceptive organs (Hustert *et al*., 1999). Table 7 lists the details, including number of pegs on each stridulatory file and the length of each file (when such structures were wholly visible).

The number of pegs of the stridulatory files of all the specimens examined (Table 7) fell within the respective numerical range reported by Grzeschik (1969) and Heller *et al*. (2022), that is 20–65 "teeth" on the stridulatory file of the left tegmen. In terms of file length, with the exception of three borderline cases, all specimens examined (Table 7) also fell within the respective file length range reported, that is, of 1.4–4.3 mm (Grzeschik, 1969; Heller *et al*., 2022). Clearly, such broad numerical ranges may render comparisons somewhat difficult. Notwithstanding, it was deemed pertinent to juxtapose measurements taken for this present study with those established by Grzeschik (1969) and Heller *et al*. (2022) to ensure a more complete investigative protocol.

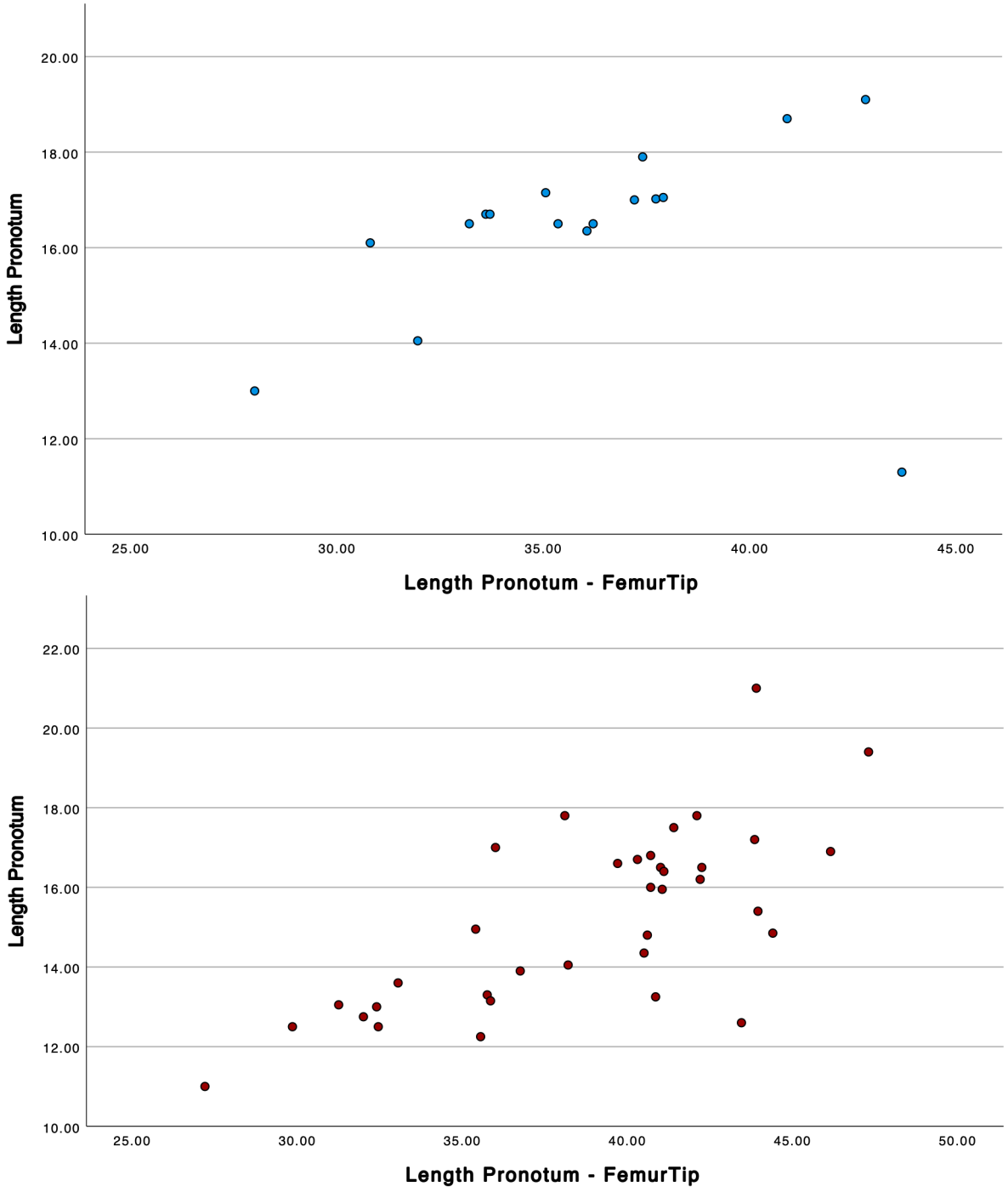

FIGURE 10 A [*top*—males (blue data points)] and **B** [*bottom*—females (red data points)]. Scatter plots showing the relationship between the length of the pronotum and the overall body length (approach based on firmly sclerotised parts of the body structure, i.e., upper rim of pronotum to the knee of the hind femur—see Methodology for rationale).

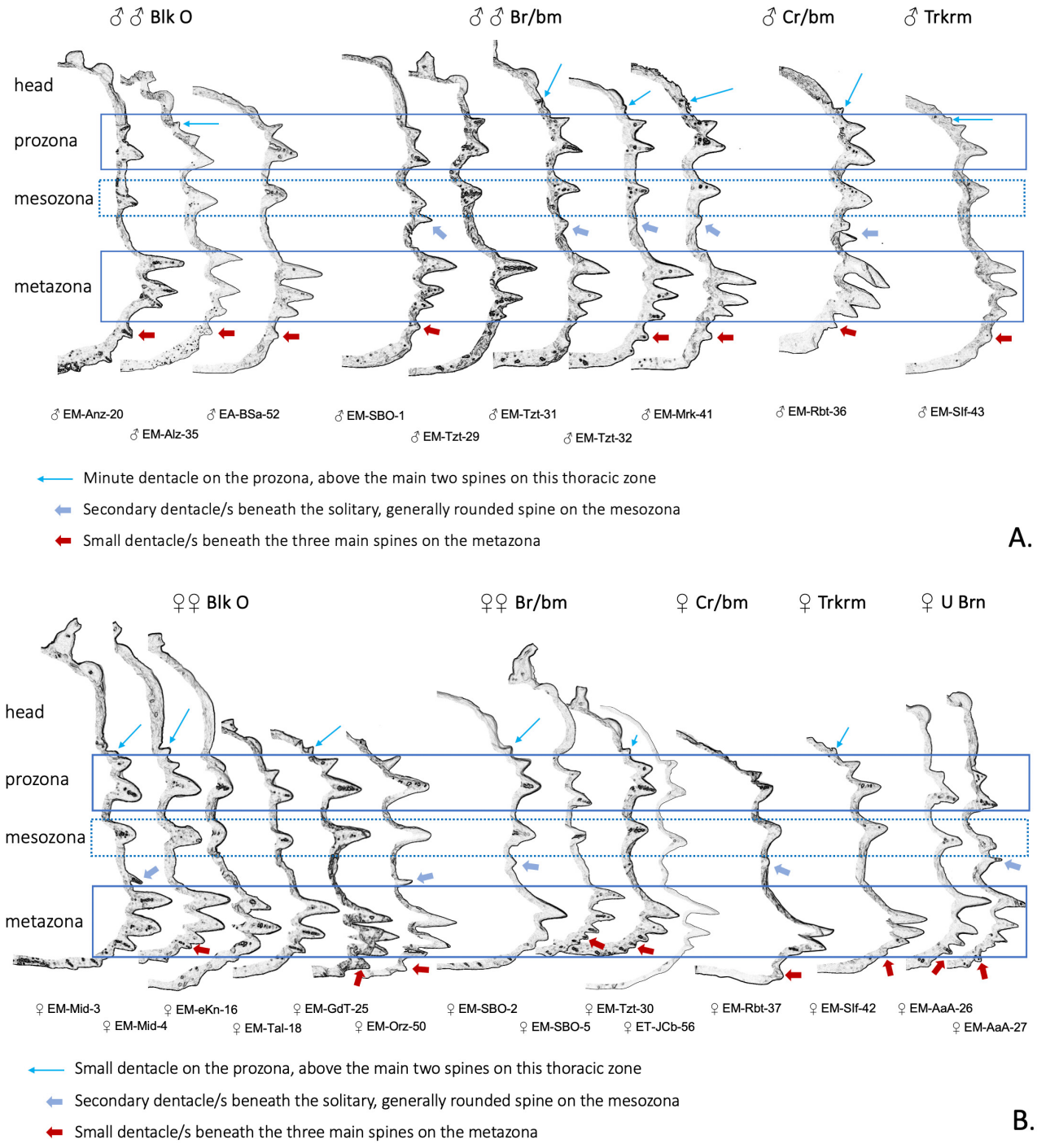

FIGURE 11 A and **B**. *Skeletised* outlines of selected males [*top*] and females [*bottom*] representing the five colour forms from different geographical locations. The general distribution of spines on all three sections of the pronotum (prozona, mesozona, metazona) is characteristically similar across the entire suite of specimens examined. Additional cuticular protuberances occur randomly in many of the representative samples of all five colour forms.

FIGURE 12. Tegmina and stridulatory files of specimens collected from either side of the Moroccan High Atlas. *Top* and *middle*: Left and right tegmina of 2♂♂ from 25 km north of Marrakech, Western Meseta, at 560 m (EM-Mrk-10) and North Tiznit, Anti-Atlas, at 210 m (EM-Tzt-32) respectively; note the much longer file on the left tegmen in either case. *Bottom*: Left tegmen and stridulatory file of ♂ collected from Anezal, Anti-Atlas, at 1550 m (EM-Anz-20).

TABLE 7. Details and measurements pertaining to the 12♂♂ specimens whose stridulatory files were examined.

Specimen code	Colour form code	Number of pegs on Left file	length of Left File	Number of pegs on Right file	length of Right File
		n	mm	*n*	mm
EM-SBO-1	Br/bm	47	3.25	33	2.30
EM-SBO-6	Br/bm	40	2.80	30	2.12
EM-SBO-7	Br/bm	41	2.72	29	1.60
EM-Mrk-10	Br/bm	39	2.30	31	2.15
EM-SBO-11	Br/bm	41	2.28	-	-
EM-Anz-12	Blk O	43	2.48	-	-
EM-Anz-20	Blk O	41	2.64	21	1.36
EM-Tzt-29	Br/bm	39	2.55	32	2.05
EM-Tzt-31	Br/bm	42	3.18	29	1.85
EM-Tzt-32	Br/bm	43	2.76	32	2.15
EM-Alz-35	Blk O	44	1.90	34	1.30

.....continued on the next page

TABLE 7. (Continued)

Specimen code	Colour form code	Number of pegs on Left file	length of Left File	Number of pegs on Right file	length of Right File
		n	mm	*n*	mm
EM-Orz-51	Blk O	35	1.50	27	1.30
Overall range:		35–47	1.50–3.25	21–34	1.30–2.30
Mean:		41.25 (*n*=12)	2.53	29.80 (*n*=10)	1.82
Colour form range:	Br/bm	39–47	2.28–3.25	29–33	1.60–2.30
	Blk O	35–44	1.50–2.64	21–34	1.30–1.36
Colour form mean:	Br/bm	41.50 (*n*=8)	2.73	30.86 (*n*=7)	2.03
	Blk O	40.75 (*n*=4)	2.13	27.33 (*n*=3)	1.32
Geographical range (scale):	Western Meseta (north of High Atlas)	39–47	2.28–3.25	29–33	1.60–2.30
	Anti-Atlas (south of High Atlas)	35–44	1.50–3.18	21–34	1.30–2.15
Geographical mean:	Western Meseta (north of High Atlas)	41.60 (*n*=5)	2.67	30.75 (*n*=4)	2.04
	Anti-Atlas (south of High Atlas)	41.00 (*n*=7)	2.43	29.17 (*n*=6)	1.67

A statistical comparison of means (amplitude of variation) was performed, respectively, of the lengths of the left and right stridulatory files, and of the number of pegs that occurred on the files examined, between (i) the two main colour forms (**Blk O** and **Br/bm**) and (ii) specimens collected from either side of the High Atlas, in this case, the Western Meseta and the Anti-Atlas.

Length of stridulatory files relative to the two main colour forms: On average, the length of left stridulatory files of Br/bm individuals was found to be longer than those of Blk O individuals, respectively, with values of 2.73 (*SD* .358) and 2.13 (*SD* .527). This trend is similarly reflected in the lengths of the right file. Br/bm individuals were found to have an average of 2.03 (*SD* .234), while Blk O individuals were found to have an average of 1.32 (*SD* .035).

Levene's test was used to verify if both samples have equal variances (Levene, 1960). For the left stridulatory file length parameter, the test showed an *F* value of 1.681 with a significance of .224, while for the right stridulatory file length parameter, the test indicated an *F* value of 3.272 with a significance of .108. In both cases, the *p*-value was > .05, indicating no statistically significant differences between the variances of the two samples.

The Independent Samples *t*-Test was then used to assess if there was statistical significance in the difference between the means of the two main colour forms (**Blk O** and **Br/bm**). The *t*-test for the left stridulatory file length resulted in a value of −2.355, with a 2-tailed *p*-value of .040 (Table 8), while for the right file length, the *t*-test was of −5.077, with a corresponding 2-tailed *p*-test of less than .001. In both cases, the results indicate a **statistically significant difference** between stridulatory file lengths of the two main colour forms.

TABLE 8. Results for *t*-test based on the length of the left stridulatory file of the two main colour forms.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Length of left file	Equal variances assumed	1.681	.224	-2.355	10	.020	.040	-.60000	.25478	-1.16768	-.03232
	Equal variances not assumed			-2.053	4.447	.051	.102	-.60000	.29226	-1.38028	.18028

Length of stridulatory files relative to geographical locations: On average, the left stridulatory file lengths of individuals from the Western Meseta were found to be longer than those of individuals from the Anti-Atlas, with respective values of 2.67 (*SD* .402) and 2.43 (*SD* .560). This trend is also reflected in the lengths of the right file, where Western Meseta individuals averaged 2.04 (*SD* .305), while Anti-Atlas individuals were found to have an average of 1.67 (*SD* .394).

Levene's Test was also carried out to check if both samples have the same variance. For the left file length parameter, the test shows an *F* value of .443 and a significance of .521. For the right file length parameter, the test shows an *F* value of 2.33 with a significance of .165. In both cases, the significance is > .05, indicating no statistically significant difference between the variances of the two samples.

The Independent Samples *t*-Test was then performed to assess if there was statistical significance in the difference between the means of the Western Meseta and the Anti-Atlas samples. The *t*-test for the left stridulatory file length produced a result of −.816, with a 2-tailed *p*-value of .434 (Table 9). For the right stridulatory file length, the *t*-test was of −1.595 and the corresponding 2-tailed *p*-test was .149. In both cases, the results are therefore **not significant**.

TABLE 9. Results for *t*-test of the length of the left stridulatory file in relation to geographical location.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Length of left file	Equal variances assumed	.443	.521	−.816	10	.217	.434	−.24000	.29411	−.89533	.41533
	Equal variances not assumed			−.865	9.985	.204	.407	−.24000	.27741	−.85824	.37824

Number of pegs on the stridulatory files relative to the two main colour forms: On average, the pegs on the left file of Br/bm individuals were found to be similar in number to those of Blk O counterparts (≈ 41). The average number of pegs on the right file of Br/bm individuals was 31 while on Blk O specimens 27 (*SD*, respectively, was 1.574 and 6.506).

As in the case of the file length, Levene's Test was carried out to investigate whether the two main colour form samples have the same variance. For the number of pegs on the left file, the test showed an *F* value of .750 with a significance of .407, indicating no statistically significant difference between the variances of the two samples. For the pegs on the right file, the test generated an *F* value of 5.842 with a significance of .042. In this case, the *t*-test was not carried out in view of a significance of < .05, indicative of a statistically significant difference between the variances of the two samples.

Subsequently, the Independent Samples *t*-Test, used to assess if there is any statistical significance in the difference between the means of the number of pegs on the left file in the two main colour forms, resulted in a value of −.394, with a 2-tailed *p*-value of .702 (Table 10). There is therefore **no significant** difference between the two main colour forms, Blk O and Br/bm.

TABLE 10. Results for t-test performed on the number of pegs of the left stridulatory file of the two main colour forms.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Number of pegs on left file	Equal variances assumed	.750	.407	−.394	10	.351	.702	−.750	1.905	−4.994	3.494
	Equal variances not assumed			−.338	4.317	.376	.751	−.750	2.218	−6.734	5.234

Number of pegs on the stridulatory files relative to geographical locations: Regardless of colour form, the number of pegs on the left file of specimens from both the Western Meseta and Anti-Atlas was, on average, ≈ 41, while the number of pegs on the right stridulatory file was also similar (≈ 30) for both sample groups on either side of the High Atlas.

As in previous cases, Levene's Test was performed to check whether the two geographical samples have equal variances. For the number of pegs on the left stridulatory file parameter, the test showed an *F* value of .012 and a significance of .914, while for the number of pegs on the right file parameter, the test generated an *F* value of 2.44 with a significance of .157. In both cases, the significance is > .05, indicating no significant difference between the variances of the two samples.

The Independent Samples *t*-Test was again used to assess if there was statistical significance in the difference between the means of the specimens from the Western-Meseta and the Anti-Atlas. The *t*-test for the number of pegs on the left stridulatory file resulted in −.329, with a 2-tailed *p*-value of .749 (Table 11). For the number of pegs on the right file, the *t*-test produced a result of −.634 with the corresponding 2-tailed *p*-test of .544. In both these cases, the results are **not significant** (accepting the null hypothesis), indicating no statistical difference between the two geographical sub-groups in terms of the number of pegs on both stridulatory files.

TABLE 11. Results for *t*-test based on the number of pegs of the left stridulatory file in relation to geographical location.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance		Mean	Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Difference	Difference	Lower	Upper
Number of pegs on left file	Equal variances assumed	.012	.914	-.329	10	.375	.749	-.600	1.826	-4.668	3.468
	Equal variances not assumed			-.328	8.732	.375	.750	-.600	1.828	-4.754	3.554

Genital sclerite structure examination—The two ♂♂ specimens (spec. codes: EM-Alz-35 and EM-Orz-51) that were examined in the conventional manner, using KOH solution to dissolve soft tissue, did not yield any structure that resembled, even remotely, the titillators of a Tettigoniid. The other four ♂♂ specimens, selected from either side of the High Atlas Mountain range (spec. codes: EM-SBO-7, EM-Mrk-10, EM-Anz-12, and EM-Tzt-29), were examined using a commercially available 'relaxing fluid', and tepid water in which drops of antiseptic were added to discourage the subsequent formation of mould. As indicated in the Methodology above, abstaining from the use of KOH has the advantage of preserving non-sclerotized tissue; in the event that *Eugaster* titillators are so constituted, as suggested by Heller *et al*. (2022), such approach would have assured the preservation of these copulatory appendices. Conversely, however, this also meant that all other material present, including other anatomical structures and undigested organic matter, formed a conglomerated mass, which presented various challenges in this aspect of the investigation, not least in distinguishing the various anatomical characters from one another. Although some structures that bear a strong resemblance to Tettigoniid titillators were observed within the mass of organic material examined belonging to both specimens that were softened with tepid water (Figure 13), it could not be incontrovertibly ascertained that these were indeed the copulatory appendices being searched for. At this juncture, it may be premature to assume the presence of such structures based on the examination of dry specimens, given their condition, and further investigations may be warranted, using freshly collected individuals.

Molecular and Phylogenetic analyses—The quality of dried specimens used varied considerably and, as a result, DNA degradation did not allow the amplification of most sequences for each specimen. Only the mitochondrial cytochrome c oxidase I (COI) marker was amplified successfully for specimens EM-AaA-27 (U Brn colour form) and EM-Anz-22 (Blk O colour form). Although this data is not comparable to the nuclear data presented in Grzywacz *et al*. (2015), it is however suitable for differentiating species, particularly among insect groups (Zhang and Bu, 2022). Reported herein are the first COI sequences of *Eugaster spinulosa*. The BLAST searches did not result in any close hits. The consensus tree for the COI data shows that the sequence for the specimens EM-AaA-27 and EM-Anz-22 clustered together and was highly supported (Figure 14: 1.00 and 100%).

Another noteworthy aspect concerning the methodologies employed for the present DNA analyses is that the specimens used were collected between 1986 and 2022, including two specimens (EM-AaA-27 and EM-Anz-22), collected in 2015 and 2022 respectively. In a recent study, Mullin *et al*. (2023) noted that most DNA fragments from old museum insect specimens were below 100 bp, even for samples collected as recently as 2004, which shows that DNA fragmentation occurs rapidly after dry preservation of the insect specimens.

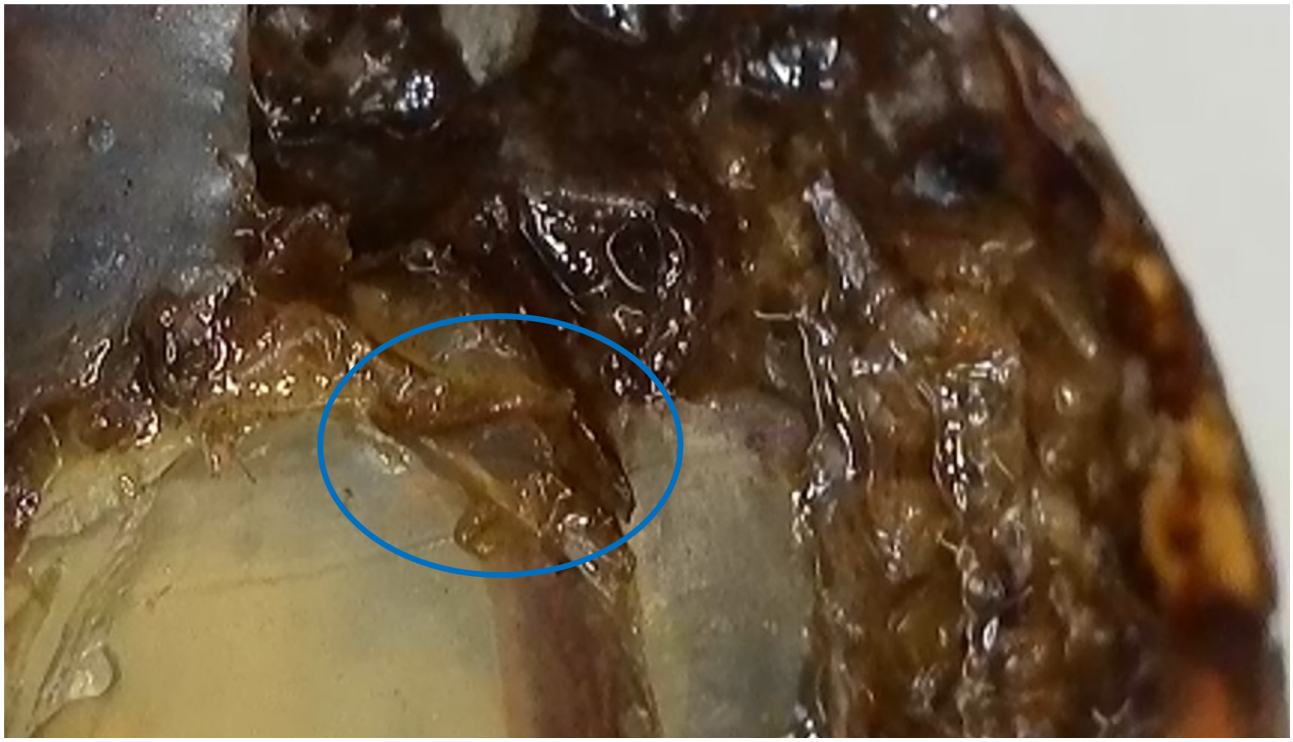

FIGURE 13. A titillator-like appendage observed during examination of the terminalia of a male from Sidi Bou-Othmane on the Western Meseta (spec. code: EM-SBO-7).

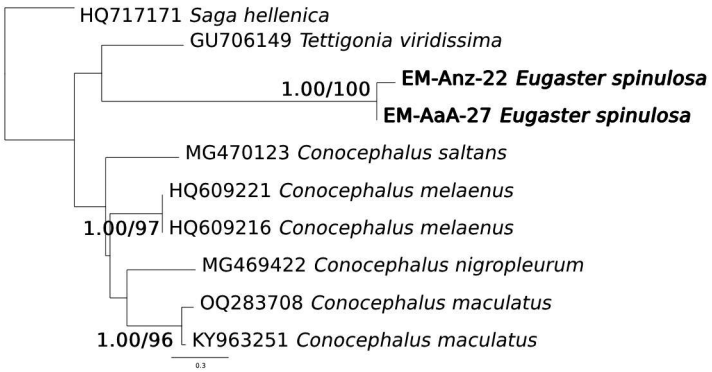

FIGURE 14. Consensus phylogenetic tree of species inferred from COI sequences. Bayesian Inference (BI) and Maximum likelihood (ML) analysis involved 9 specimens (of six species) and one outgroup taxon. The numbers on branches are Bayesian posterior probabilities (BPP) and bootstrap (BS) values (> 0.75 and 75%, respectively). The scale bar represents the number of substitutions per site.

In the present study, it was deemed pertinent to include both successful and negative outcomes since the evaluation of results involving genotyping from museum specimens has often been ambiguous, with most studies being limited only to successful results (Lalonde & Marcus, 2020). In order to heighten the chances of success, when employing the methodology outlined herein, it would be advisable to focus on fresh material in future genetic work on this genus.

Limitations in connection with DNA analyses—DNA degrades post-mortem as a function of time and heat (Lindahl, 1991) and, as has been attested, molecular-based studies have mostly been limited to recently collected specimens preserved in a specific manner for such work (Gilbert *et al*., 2007). Studies that investigated old and degraded DNA have, in general, demonstrated a characteristically high ratio of mitochondrial to nuclear template molecules, with mitochondrial DNA remaining PCR-amplifiable for longer in comparison to nuclear DNA (Lindahl,

1991). In fact, Lalonde and Marcus (2020) reported that successful works on museum specimens have mostly used either nuclear microsatellites or fragments of mitochondrial DNA, with the latter being the more successful approach. However, recent literature has also shown that some efforts were successful in recovering both nuclear and mitochondrial data from historical insect specimens, which suggests that it may be possible to characterise nuclear genetic data from a specimen where mitochondrial methods have been successful (Lalonde and Marcus, 2020). Thus, whilst nuclear DNA generally requires fresh specimen or specimen frozen at -20 °C, mitochondrial DNA is in general more obtainable from older specimen. However, noting that the only previous study with published sequences for the genus *Eugaster* used nuclear DNA from fresh material, the present authors attempted to amplify both mitochondrial and nuclear DNA.

Discussion and Conclusions

Clearly, *Eugaster* demonstrates a morphological variability so great (in terms of dimensions, coloration and shape, in particular, the thoracic structure), it is unsurprising that so many species and subspecies have been described. Few other species of Orthoptera, if any, are known to possess such a disparate morphology. The present research has established a number of findings, derived from a broad and multi-faceted approach, key among which are outcomes pertaining to:

(i) the relationship between altitude and geographical distribution of colour forms;
(ii) biometric analysis across different forms;
(iii) thoracic morphology;
(iv) stridulatory file configuration;
(v) the likely presence of non-sclerotized titillators; and
(vi) DNA sequencing and phylogenetic analyses.

Coloration is distinctly variable among populations, although in general, colour forms appear to be location-specific, with the two most common forms (overall black form with red coloration on the pronotum - Blk O, and the light-coloured brownish form with chequered abdominal markings - Br/bm) occurring on either side of the High Atlas Mountains. The range of the Blk O form (Figure 15) also extends to localities on both flanks of the Middle Atlas (Western Meseta and the High Plateau), and eastward into Algeria and Tunisia. Br/bm forms also occur on the Western Meseta and Anti-Atlas, as well as around Jebel Chaambi in western Tunisia, albeit at lower altitudes. **Note**: four Blk O specimens, collected from massifs within central-south Tunisia, were examined at the *Museo Civico di Storia Naturale di Trieste*; since individual specimen data labels lacked detailed locality information, it was decided not to include these specimens in the respective calculations. Spatial data suggests there is a clear link between topographic elevation and geographical distribution, the salient finding among which is the fact that colour form distribution patterns adhere to an altitude gradient (refer to Figure 8 and related details in the Results and Discussion section). This is potentially an adaptation to thermoregulation, although secondary factors may be the taxon's limited mobility (being apterous and relatively quite heavy), and its occurrence within habitat patches dominated by rocky steppe, fragmented by a non-traversable rugged mountainous terrain. This effectively results in reproductive barriers between sub-populations, which play a role in metapopulation dynamics due to demographic isolation. Hence, while processes consequent to vicariant events are likely to be at play as a key source of spatial separation, the supposition that geographical distribution of colour forms may be in line with the *thermal melanism hypothesis* ought to be researched further.

Biometric variance is markedly evident, not only between sexes but also among same-sex individuals of the same colour form, as well as across different colour forms. The largest Blk O female within the suite of specimens examined measured 50.20 mm, whereas the smallest male and female of the same colour form measured 30.00 mm and 31.80 mm respectively; the largest and smallest specimens of the entire sample, both females, measured 51.40 mm (Cr/bm) and 28.70 mm (U Brn), respectively. Notwithstanding this broad biometric variation, statistical analysis nonetheless revealed no significance across all parameters in the case of ♂♂; the same applies for ♀♀ except for variables involving thoracic width (with spines and excluding spines), which were significantly different between colour forms, albeit only slightly, with differences nonetheless proportional to the individual's overall dimensions (see following paragraph on thoracic morphology).

The results attained through statistical and edge detection analysis of thoracic cuticular armour morphology demonstrate an element of variability among and across all colour forms with respect to the structure of the pronotum (inclusive of the prozona, mesozona, metazona). By and large, the main protuberances (spines and denticles) appear to be characteristically uniform for all specimens examined, in terms of spatial arrangement and general structure. In fact, all 53 adult specimens examined were biometrically quite similar and, statistically, it was concluded that the thoracic length and width of each individual specimen were, as a rule, proportional to overall size. However, a random array of secondary spines can sometimes be present on some or all three divisions of the pronotum of all colour forms (Figures 11 A and 11 B). For these reasons, notably the (i) analogous biometric structural arrangement of main protuberances, and (ii) non-systematic presence of secondary spines and denticles that can occur, albeit inconsistently, in all colour forms and on individuals recorded from all geographical regions, reliance on such characters as a means for dependable and meaningful determination of taxa is highly questionable, given that these supposed defining characters (convincingly relied upon by various workers in the past) are in reality pervasive.

The stridulatory file of the two main colour forms (Blk O and Br/bm) was examined in terms of length and number of pegs (Figure 12 and Tables 7–11), with emphasis on the file of the left tegmen (though the right tegmen file was also examined); results were also compared with measurements in Grzeschik (1969) and Heller *et al*. (2022). Although both the number of pegs and length of the file on the left tegmen fell well within the stipulated range (with minimal nonconformity in respect to the length of the right tegmen file of three individual specimens), the established range for both length and number of pegs remains much too broad for meaningful comparisons. In terms of statistical significance or otherwise, only the length of the stridulatory files of the two main colour forms proved to be statistically significant. On the other hand, the following three variables were not significant: (i) the length of stridulatory files relative to geographical location (both tegmina); (ii) the number of pegs relative to the two main colour forms (left tegmina); and (iii) the number of pegs relative to geographical location (both tegmina). Thus, this indicates no statistical difference between the two geographical sub-groups (from the Western Meseta and the High Plateau) in terms of the length and number of pegs on both stridulatory files, as well as the number of pegs on the left tegmen of the Blk O and Br/bm colour forms.

On the presence of titillators, although structures very similar to such Tettigoniid appendices were observed in some of the specimens examined without the use of KOH (therefore occurrence cannot be ruled out), much remains to be seen with regard to the precise nature and extent to which these genital structures are sclerotized. A reliable way to determine this could be through the employment of a 3-D imaging technique known as microtomography (or Micro-CT). A μCT scan would provide a high-resolution X-ray image of the entire genital sclerite structure, literally exposing a slice-by-slice internal view for detailed investigation. This procedure should ideally be carried out on fresh specimens that have not been eviscerated for eventual preservation, or even on live individuals. A classic and well-defined example of this approach is demonstrated in Eberhard & Lehmann (2019), wherein a pair of *Roeseliana roeselii* (Hagenbach, 1822), were scanned while copulating.

As regards the molecular and phylogenetic analyses, the consensus phylogenetic tree for the COI gene (Figure 14) involving two specimens from the Anti-Atlas but belonging to two different colour forms—EM-AaA-27 (U Brn), collected from Aarba ait Ahmed at 360 m AMSL and EM-Anz-22 (Blk O), collected from Anezal at 1550 m AMSL—did not result in separate clustering. Consequently, the phylogenetic analysis supports that aspect of the morphometric analyses carried out in the present study, which demonstrated that the different colour forms do not correspond to different taxa. Since, however, the use of single organelle markers could potentially overlook interspecific mitochondrial introgressions (Hawlitschek *et al*., 2017; Bartolo *et al*., 2020; Geiger *et al*., 2021; Zhao *et al*., 2022), it is proposed that a future study analyses both uniparental (e.g. COI) and biparental (nrRNA genes) inherited DNA from a larger sample size of fresh specimens.

In summary, the foregoing results of this investigation point towards a morphology that is exceptionally variable, possibly only surpassed by tegmen and hindwing variability exhibited by Neotropical katydid species, namely *Pterochroza ocellata* (Linneaus, 1758) and *Typophyllum trapeziforme* (Stoll, 1813) [Tettigoniidae, Pterochrozinae], the many forms of which are discussed at length and illustrated in Xiberras & Ducaud (2020). As indicated, findings suggest that *Eugaster* morphology, including that of the thoracic structure, notwithstanding a seemingly high degree of variance within and between colour forms, is essentially analogous. Assigning names to colour variants or on the basis of morphological characters would therefore seem futile since there are no tangible and distinct morphological boundaries that can be quantifiably separated with reasonable confidence. Moreover, on the issue of colour form distribution, perhaps one of the foremost findings is that geographical distribution of the various colour variants follows an altitude gradient, highlighted in Figure 8.

FIGURE 15. ♀ Blk O specimen at a locality SW of Aït Melloul (Anti-Atlas), *alt*. >1000 m, on 29.iii.2019 (Photo: P. Fontana).

Despite various past endeavours to separate taxa on the basis of coloration, thoracic structure and size, the fact remains that environmental conditions, influenced by prevailing weather in a given year or a period of a few years, can have a meaningful impact on habitat factors and on the availability of food sources, which will in turn affect processes involving growth and development during metamorphosis. It is also well-established that seasonal polyphenism is common in some insect groups, where seasonal weather conditions lead to diverse phenotypes, both periodically and cyclically (Samoilov *et al*., 2006; Simpson *et al*., 2011; Yang & Pospisilik, 2019; Rodrigues & Beldade, 2020). Perturbations caused by temperature and other variables, including stochastic fluctuations, in a particular year can lead to changes, mostly influencing size in *Eugaster*, through phenotypic plasticity; this is evident by the broad range of specimen sizes from common localities (Table 12). Thus, notwithstanding the taxon's perceptible susceptibility to exposure to the elements, phenotypic plasticity seems to afford *Eugaster* the capacity to adapt to local or short-term environmental stress. The consequential response to altitude gradient, through colour form expression, is yet another impact of plasticity.

Organisms with non-overlapping distributions that can interbreed freely and produce viable offspring are generally classified as separate taxa in concurrence with the biological species concept. It may also seem unlikely that, in case of sympatry, recently diverged taxa would interbreed mostly due to enhanced mate discrimination (Coyne & Orr, 1989; Howard, 1993). The preference to mate with individuals of a similar phenotype will tend to enhance the genetic traits specific to a sub-population and, as a result, reinforce morphological characters, including colour form variation. Such 'assortative mating', coupled with topographic barriers (e.g. altitude gradient that tends to maintain a degree of separation between *Eugaster* colour forms) could effectively impede crossbreeding between different phenotypic variants. As a consequence, this restricts gene flow and reinforces the separation between colour forms, while continuing to bolster the isolation process between sub-populations occupying a patchy spatial arrangement within a highly fragmented landscape mosaic typical of the mountainous Maghreb, the dynamics of which could contribute to genetic divergence. In addition to maintaining a distinct genetic make-up, subspecies comprise biological entities that represent a characteristic natural history, also distinct from that of other taxa of similar taxonomic rank (O'Brien & Mayr, 1991). In the case of *Eugaster*, the rank of subspecies has been assigned by several authors in the past, even where the geographical range of some sub-populations was spatially unclear or somewhat hazy due to the potential of range boundary overlap. As is evident from findings in the present work, colour form variation is strongly associated with elevation, while numerous other morphological characters, for instance the general shape and position of some thoracic protuberances, spines and denticles (previously considered

fundamental in identification keys), either occur in all variants or occur infrequently across all colour variants. Such findings imply clinal variation or a dependency on other environmental factors, including food sources (elaborated above). Consequently, when morphological characters within and between sub-populations exhibit variance which lacks dependable and predictable uniformity (for the purpose of comparison and contrast), such characters are rendered effectively unsuitable as a basis for reliably distinguishing between taxa. This is especially the case when subspecific ranking has been assigned to groups with potentially overlapping geographical distribution; in these instances, consideration should be given to synonymise these taxa and simply assign names to distinguish between geographical variants.

TABLE 12. Appendix with field data, assigned codes, morphometric measurements and other details of all specimens examined for the present study.

Specimen code EM = Morocco EA = Algeria ET = Tunisia	**Specimen Data** MSNG: *Museo Civico di Storia Naturale*, Genoa; NMNH: National Museum Natural History, Malta; BMPC: B. Massa, private collection; LFCPC: L-F Cassar, private collection; ACPC: A. Catania, private collection	Colour form code	Length: Head + Pronotum	Length: Pronotum	Length: Head—Femur tip	Thorax width + spines	Thorax width excl. spines
EM-SBO-1 *alt.* 516 m	♂ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Catania (ACPC) Stridulatory file extracted.	Br/bm	19.70	17.00	39.90	16.60	9.22
EM-SBO-2 *alt.* 516 m	♀ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Catania (ACPC)	Br/bm	19.85	17.20	46.50	20.20	12.36
EM-Mid-3 *alt.* 1677 m	♀ Midelt (High Plateau), 27.vi.2006; *leg.* A. Seguna (NMNH)	Blk O	18.75	13.90	41.60	17.20	9.85
EM-Mid-4 *alt.* 1677 m	♀ Midelt (High Plateau), 27.vi.2006; *leg.* A. Seguna (NMNH)	Blk O	20.05	14.05	44.20	17.55	9.20
EM-SBO-5 *alt.* 516 m	♀ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Seguna (LFCPC) DNA extraction.	Br/bm	21.40	15.95	46.50	18.65	10.80
EM-SBO-6 *alt.* 516 m	♂ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Seguna (NMNH) Stridulatory file extracted.	Br/bm	15.72	11.30	48.10	17.10	10.45
EM-SBO-7 *alt.* 516 m	♂ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Seguna (LFCPC) Stridulatory file extracted; terminalia examined.	Br/bm	21.50	19.10	45.20	18.05	10.75
EM-Mid-8 *alt.* 1677 m	♀ Midelt (High Plateau), 27.vi.2006; *leg.* A. Seguna (LFCPC) DNA extraction.	Blk O	18.20	12.25	41.50	16.55	9.75
EM-Set-9 *alt.* 252 m	♀ Settat env., south of Casablanca (Western Meseta), 15-21.viii.2015; *leg.* A. Abramov (LFCPC) DNA extraction.	Blk O	16.65	13.30	39.10	16.75	9.70
EM-Mrk-10 *alt.* 560 m	♂ 25 km north of Marrakech (Western Meseta), 15.v.2015; *leg.* M. Garrod (LFCPC) Stridulatory file extracted; terminalia examined.	Br/bm	18.60	17.02	39.30	16.30	9.60
EM-SBO-11 *alt.* 516 m	♂ Sidi Bou-Othmane (Western Meseta), 22.vi.2006; *leg.* A. Seguna (LFCPC) Stridulatory file extracted.	Br/bm	20.25	17.05	41.10	16.95	11.05
EM-Anz-12 *alt.* 1550 m	♂ Anezal (Anti-Atlas), 07.vi.2022; leg. L. Saltini (LFCPC) Stridulatory file extracted; terminalia examined.	Blk O	14.95	14.05	32.85	14.40	8.65
EM-Anz-13 *alt.* 1550 m	♀ Anezal (Anti-Atlas), 07.vi.2022; leg. L. Saltini (LFCPC)	Blk O	17.40	13.15	40.10	16.90	9.00
EM-Anz-14 *alt.* 1550 m	♀ Anezal (Anti-Atlas), 07.vi.2022; leg. L. Saltini (LFCPC)	Blk O	15.90	12.60	46.75	18.90	10.95
EM-Anz-15 *alt.* 1550 m	♀ Anezal (Anti-Atlas), 07.vi.2022; leg. L. Saltini (LFCPC) DNA extraction.	Blk O	18.80	14.35	44.95	18.10	9.10

.....continued on the next page

TABLE 12. (Continued)

Specimen code EM = Morocco EA = Algeria ET = Tunisia	**Specimen Data** MSNG: *Museo Civico di Storia Naturale*, Genoa NMNH: National Museum Natural History, Malta BMPC: B. Massa, private collection LFCPC: L-F Cassar, private collection ACPC: A. Catania, private collection	Colour form code	Length: Head + Pronotum	Length: Pronotum	Length: Head—Femur tip	Thorax width + spines	Thorax width excl. spines
EA-eKn-16 *alt.* 710 m	♀ El Kantara, Jebel Metlili (Aurès mountain range), x.1986; leg. L-F. Cassar (LFCPC) DNA extraction.	Blk O	20.95	16.90	50.20	20.70	12.40
EM-Tal-17 *alt.* 1425 m	♀ Taliouine env., Souss-Massa region (Anti-Atlas), 25.ii.2015; leg. A. Abramov (LFCPC)	Blk O	15.40	12.75	34.65	15.10	8.95
EM-Tal-18 *alt.* 1425 m	♀ Taliouine env., Souss-Massa region (Anti-Atlas), 25.ii.2015; leg. A. Abramov (LFCPC) DNA extraction.	Blk O	14.45	12.50	31.80	16.10	9.60
EM-Tal-19 *alt.* 1425 m	♀ Taliouine env., Souss-Massa region (Anti-Atlas), 25.ii.2015; leg. A. Abramov (LFCPC)	Blk O	14.80	13.05	33.00	17.30	10.35
EM-Anz-20 *alt.* 1550 m	♂ Anezal (Anti-Atlas), 07.vi.2022; DNA extraction. leg. Pad Mal (LFCPC) Stridulatory file extracted.	Blk O	18.40	16.50	38.10	19.85	8.40
EM-Anz-21 *alt.* 1550 m	♀ Anezal (Anti-Atlas), 07.vi.2022; leg. Pad Mal (LFCPC)	Blk O	17.40	14.85	46.95	19.80	9.70
EM-Anz-22 *alt.* 1550 m	♀ Anezal (Anti-Atlas), 07.vi.2022; leg. L. Saltini (LFCPC) DNA extraction.	Blk O	19.65	14.80	45.45	17.90	9.45
EM-Alz-23 *alt.* ≈1250 m	♀ Aoulouz (Anti-Atlas), 16.vi.1998; leg. Daniele Sechi (LFCPC)	Blk O	20.95	16.50	46.65	18.05	9.70
EM-GdT-24 *alt.* 1795 m	♀ Gorges du Todrha (High Atlas—Anti-Atlas interface), 11.vii.2012; leg. I Zappi (LFCPC)	Blk O	18.00	15.40	46.55	18.25	10.00
EM-GdT-25 *alt.* 1795 m	♀ Gorges du Todrha (High Atlas—Anti-Atlas interface), 11.vii.2012; leg. I Zappi (LFCPC) ***note:*** abdomen distorted DNA extraction.	Blk O	14.45	12.50	34.40	13.20	8.55
EM-AaA-26 *alt.* 360 m	♀ Aarba ait Ahmed (Anti-Atlas), 15.v.2015; leg. G L Agnoli (LFCPC) DNA extraction.	U Brn	18.40	14.95	38.85	17.30	9.65
EM-AaA-27 *alt.* 360 m	♀ Aarba ait Ahmed (Anti-Atlas), 15.v.2015; leg. G L Agnoli (LFCPC) ***note:*** in relatively poor condition. DNA extraction.	U Brn	21.50	16.20	47.50	17.60	9.20
EM-Tal-28 *alt.* 1483 m	♀ Taliouine env., Souss-Massa region (Anti-Atlas), 04.vi.2013; leg. G L Agnoli (LFCPC)	Blk O	17.55	13.60	37.00	16.30	9.80
EM-Tzt-29 *alt.* 210 m	♂ North Tiznit (Anti-Atlas), 15.v.2015; leg. M. Garrod (LFCPC) Stridulatory file extracted; terminalia examined.	Br/bm	17.35	16.35	37.05	16.45	9.00
EM-Tzt-30 *alt.* 210 m	♀ North Tiznit (Anti-Atlas), 15.v.2015; leg. M. Garrod (LFCPC) DNA extraction.	Br/bm	14.85	13.25	42.45	17.55	10.35
EM-Tzt-31 *alt.* 210 m	♂ North Tiznit (Anti-Atlas), 15.v.2015; DNA extraction. leg. M. Garrod (LFCPC) Stridulatory file extracted.	Br/bm	18.25	16.50	37.10	15.15	9.60
EM-Tzt-32 *alt.* 210 m	♂ North Tiznit (Anti-Atlas), 15.v.2015; leg. M. Garrod (LFCPC) Stridulatory file extracted.	Br/bm	18.05	17.15	35.95	15.95	9.45
EM-Alz-33 *alt.* ≈1250 m	♀ Aoulouz (Anti-Atlas), 16.vi.1998, (MSNG) DNA extraction.	Blk O	22.40	17.80	46.70	20.40	13.20
EM-Alz-34 *alt.* ≈1250 m	♀ Aoulouz (Anti-Atlas), 16.vi.1998, (MSNG)	Blk O	21.4	17.0	40.4	18.4	12.0

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TABLE 12. (Continued)

Specimen code EM = Morocco EA = Algeria ET = Tunisia	**Specimen Data** MSNG: *Museo Civico di Storia Naturale*, Genoa NMNH: National Museum Natural History, Malta BMPC: B. Massa, private collection LFCPC: L-F Cassar, private collection ACPC: A. Catania, private collection	Colour form code	Length: Head + Pronotum	Length: Pronotum	Length: Head—Femur tip	Thorax width + spines	Thorax width excl. spines
EM-Alz-35 *alt.* ≈1250 m	♂ Aoulouz (Anti-Atlas), 16.vi.1998, (BMPC) Stridulatory file extracted; terminalia examined.	Blk O	20.3	17.9	39.8	17.5	10.4
EM-Rbt-36 *alt.* <95 m	♀ Rabat (Western Meseta), identified as *spinulosus*, (MSNG)	Cr/bm	23.5	19.4	51.4	23.0	14.5
EM-Rbt-37 *alt.* <95 m	♂ Rabat (Western Meseta), identified as *spinulosus*, (MSNG)	Cr/bm	21.6	18.7	43.8	19.5	10.4
EM-Azu-38 *alt.* >1200 m	♀ nymph Azrou (Western Meseta—Middle Atlas boundary), v.1998 (BMPC) ***note:*** in poor condition	Blk O	-	-	-	-	-
EM-Azu-39 *alt.* >1200 m	♀ nymph Azrou (Western Meseta—Middle Atlas boundary), v.1998 (BMPC) ***note:*** in poor condition	Blk O	-	-	-	-	-
EM-Mrk-40 *alt.* 516 m	♂ 25 km N Marrakech (Western Meseta), 22.v.2014, (MSNG)	Br/bm	18.8	16.1	33.5	15.3	9.1
EM-Mrk-41 *alt.* 516 m	♂ 25 km N Marrakech (Western Meseta), 22.v.2014, (MSNG)	Br/bm	18.8	16.7	35.8	16.4	10.0
EM-SIf-42 *alt.* <15 m	♀ Sidi Ifni (Anti-Atlas), vi.1934, *Eugaster fernandezi* paratypus, (MSNG)	Trkrm	18.0	16.8	41.9	18.8	11.9
EM-SIf-43 *alt.* <15 m	♂ Sidi Ifni (Anti-Atlas), vi.1934, *Eugaster fernandezi* paratypus, (MSNG)	Trkrm	17.8	16.5	34.5	15.2	10.0
EM-Taz-44 *alt.* ≈1510 m	♀ 20 km N Taznakht (Anti-Atlas), 21.v.2015 (identified as prope *fernandezi*) (MSNG)	Blk O	20.8	16.7	44.4	19.2	11.4
EM-Taz-45 *alt.* ≈1510 m	♀ 20 km N Taznakht (Anti-Atlas), 21.v.2015 (identified as prope *fernandezi*) (MSNG)	Blk O	20.5	16.5	45.0	21.0	11.6
EM-Taz-46 *alt.* ≈1510 m	♀ 20 km N Taznakht (Anti-Atlas), 21.v.2015 (identified as prope *fernandezi*) (BMPC)	Blk O	19.2	16	43.9	19.3	10.7
EM-Taz-47 *alt.* ≈1510 m	♀ 20 km N Taznakht (Anti-Atlas), 21.v.2015 (identified as prope *fernandezi*) (BMPC)	Blk O	19.7	17.8	40.0	19.0	11.2
EM-Alz-48 *alt.* ≈1250 m	♀ Aoulouz (Anti-Atlas), 16.vi.1998, (MSNG)	Blk O	23.8	21.0	46.7	20.4	11.0
EM-Orz-49 *alt.* 1450 m	♀ Ouarzazate, Drâa-Tafilalet (Anti-Atlas), 37 km from Taznakht, 23.v.2023; leg. M. Romano (BMPC)	Blk O	18.5	16.6	41.6	19.6	11.0
EM-Orz -50 *alt.* 1450 m	♀ Ouarzazate, Drâa-Tafilalet (Anti-Atlas), 37 km from Taznakht, 23.v.2023; leg. M. Romano (BMPC)	Blk O	14.4	13.0	33.8	16.2	9.9
EM-Orz-51 *alt.* 1450 m	♂ Ouarzazate, Drâa-Tafilalet (Anti-Atlas), 37 km from Taznakht, 23.v.2023; leg. M. Romano (BMPC) Stridulatory file extracted; terminalia examined.	Blk O	15.0	13.0	30.0	14.6	8.5
EA-BSa-52 *alt.* ≈700 m	♂ Bou Saada (Ouled Naïl, Saharan Atlas), (identified as *guyoni* var. *lucasii*) (MSNG)	Blk O	18.2	16.7	35.1	16.7	9.6
ET-Tmz-53 *alt.* 10 m	♀ Tamerza (Aurès mountain range), v.1873 (identified as *guyoni*) (MSNG)	U Brn	12.5	11.0	28.7	12.5	8.2
ET-Tmz-54 *alt.* 10 m	♂ nymph Tamerza (Aurès mountain range), v.1873 (identified as *guyoni*) (MSNG) ***note:*** in poor condition	U Brn	-	-	-	-	-

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TABLE 12. (Continued)

Specimen code EM = Morocco EA = Algeria ET = Tunisia	**Specimen Data** MSNG: *Museo Civico di Storia Naturale*, Genoa; NMNH: National Museum Natural History, Malta; BMPC: B. Massa, private collection; LFCPC: L-F Cassar, private collection; ACPC: A. Catania, private collection	Colour form code	Length: Head + Pronotum	Length: Pronotum	Length: Head—Femur tip	Thorax width + spines	Thorax width excl. spines
ET-Tmz-55 *alt.* 10 m	♀ nymph Tamerza (Aurès mountain range), v.1873 (identified as *guyoni*) (MSNG) ***note:*** in poor condition	U Brn	-	-	-	-	-
ET-JCb-56 *alt.* ≈490 m	♀ Jebel Chaambi (Aurès mountain range), 5.iv.2002, (BMPC)	Br/bm	21.5	17.5	45.4	18.7	12.3
ET-JCb-57 *alt.* ≈490 m	♀ Jebel Chaambi (Aurès mountain range), 5.iv.2002, (BMPC)	Br/bm	20.0	16.4	44.7	19.5	11.6
Additional specimen used solely for molecular and phylogenetic analyses							
EM-Mrk *alt.* 475 m	♀ 30 km N Marrakech (Western Meseta), v.2014, (LFCPC) DNA extraction.	Br/bm	Laboratory code number: 13; specimen was acquired when the current research was well under way.				

Since sample size is not generally indicated in some of the early works, initial keys may also be susceptible to limitations associated with extrapolability for meaningful comparative purposes. Moreover, the reliance on hand-drawn line diagrams may depart from an exact derivation of form, since accuracy will depend considerably on the individual's art skills, on one's individual focus in depicting morphological characters and, to some degree, on artistic licence. The real-life images used to measure thoracic morphology in the present work eliminate such potential discrepancy, thus reducing the margin of error to practically nil.

Notwithstanding the necessity of analysing more genes from a larger sample of freshly collected specimens, in view of the morphometric analysis results presented in this paper, it is highly unlikely that further biomolecular data will produce a different outcome. Indeed, this study has shown that the array of diagnostic characters used in the past to describe a suite of species within the genus *Eugaster* has been misleading largely due to the numerous inconsistencies rendered through the present research and that the various taxa previously characterised at species level are no more than infraspecific variants or forms. As a consequence, the authors propose to synonymise *Eugaster guyoni* (Serville, 1838) with *Eugaster spinulosa* (Johannson, 1763); thus, based on foregoing results, the genus *Eugaster* is represented in North Africa by a single but highly variable species, in terms of size, coloration and thoracic morphology.

Declaration of roles and of non-conflict of interest—L-F.C. and B.M. conceived the study and realized specimen acquisition, sampling, laboratory preparations for morphometric taxonomy, and biometric examinations. A.G. performed all statistical tests and prepared the images for the process involving feature extraction and edge detection. A.G.B. and S.S. performed DNA extractions, DNA amplifications, and sequencing. L-F.C. and B.M. drafted the manuscript; A.G.B. drafted the sections on molecular and phylogenetic analyses. All authors edited and approved the manuscript. The authors declare that they have no conflict of interest.

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