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Some techniques for the study of useful characters in the taxonomy of the genus *Timarcha* Samouelle, 1819 (Coleoptera, Chrysomelidae)

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Abstract. Historically the taxonomy of the genus *Timarcha* has been, and continues to be, quite confusing and largely erroneous. The confusion is mainly due to the absence of reliable traits that aid in precise identification; the lack of study of types at species-level has also contributed to the difficulty in the taxonomy. To improve this situation, we propose techniques for the dissection and study of three useful diagnostic characters such as the vestiture of the sole of female tarsi, and the morphology of the endophallus and its sclerites in males. These features combined are distinctive for each of the species and can help to resolve the taxonomy of the genus.

Keywords. Tarsi, sclerites, endophallus, dissection, Chrysomelinae.

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Introduction

The species belonging to the genus *Timarcha* are distributed in two disjunct geographical areas. The greater part of the species lives in the Western Palaearctic region, including Europe, North Africa and Asia Minor (Gómez-Zurita & Kippenberg 2010), where two subgenera, *Timarcha* s. str. and *Metallotimarcha* Motschulsky, 1860, are found. Two other species belonging to subgenus *Americanotimarcha* Jolivet, 1948 are spread out along Vancouver Island and British Columbia (Canada) and Washington, Idaho, Oregon and California (western coast of U.S.A.) (Riley *et al.* 2003).

Timarcha is considered to be a highly diverse genus in the Western Palaearctic (Warchałowski 2010). Weise (1916) recognized 67 species-group names. Jolivet (1945) proposed the sum of the Palaearctic species to be approximately 90–100. Between 1946 and 1962, Bechyně described 125 species-group names (Seeno *et al.* 1976). More recently, Gómez-Zurita (2008) recorded 316 proposed names at the specific or infraspecific level, while 204 species-group names are listed as valid in the Palaearctic catalogue (Gómez-Zurita & Kippenberg 2010).

The taxonomy of the genus is confusing; a number of species-group taxa are insufficiently described (Petitpierre 1970; Tiberghien 1971; Kippenberg 2010) or their current concepts do not match their extant types (Daccordi & Vela, unpubl. data). Old descriptions are mostly based on variable characteristics such as sculpture or size. An interesting example are the *Timarcha* from the island of Corsica. Bechyně (1944), in an extreme mania of splitting, without the methodological precaution of searching for and examining pre-existing types, described four species-level taxa closely related to each other in the *T. sardea* group (i.e., *T. cornuta* Bechyně, 1944, *T. zavadili* Bechyně, 1944, *T. sardea* ssp. *corsica* Bechyně, 1944 and *T. susterai* Bechyně, 1944). At that time, *T. prunneri* Herrich-Schaeffer, 1838, *T. sardea* (Villa & Villa, 1835), *T. sicedilis* Reiche, 1860 and *T. sublaevis* Fairmaire & Allard, 1873, belonging to the same group, had been described. Unsurprisingly, it has not been possible to find specific differences between all these taxa (Daccordi & Vela, unpubl. data).

There are various possibilities which could explain the current confusion in the taxonomy of this genus. First and foremost, past authors have never studied the types of the species-group names. In addition, many of the species show a high variability in some traits considered to be of diagnostic value in other Chrysomelidae, such as the shape of the maxillary palps, pronotum and elytra (Tiberghien 1971), size, coloration, punctuation and elytral sculpture, morphology of aedeagi and spermatheca. The case of *T. balearica* Gory, 1833 is paradigmatic for coloration variability, with specimens being blue, violet, green, black, or sometimes a combination of two of these colours. Notwithstanding, *T. marginicollis* Rosenhauer, 1856 can be black or bluish, however, in populations from Albacete: Sierra de Alcaraz, Spain, they appear purple or even golden (forma *splendida* Pérez-Arcas, 1872). A lesser but still notable colour variability is present in *T. goettingensis* (Linnaeus, 1758), which may be bluish or black. Since the knowledge of the geographic source of the specimens has been one of the most powerful diagnostic tool for species or subspecies identification in most occasions a more "topological" than "typological" concept has been applied (Gómez-Zurita 2008). This phenomenon does not occur in other Palaearctic Chrysomelidae genera.

Therefore, a re-evaluation of features that can characterize each particular species is needed. Each of the names at species-group level should also be checked to establish appropriate synonyms and stabilize the nomenclature and the taxonomy of this genus. *Timarcha* is not only problematic at species level, but also in the higher classification, ranging from the classically assigned tribe level Timarchini (Gómez-Zurita & Kippenberg 2010) to a subfamily level, Timarchinae, which could be a sister group of Galerucinae + Chrysomelinae (Gómez-Zurita *et al.* 2008; Song *et al*. 2017).

In this article, we describe the techniques for the observation and study of some characters which may shine light on the taxonomy of the genus, as some authors have already recognized. The proposed techniques will help with the study of the following three characters: the vestiture of the sole of the female tarsi, along with the morphology of the endophallus and its sclerites in males. The vestiture of the sole of the tarsi as a diagnostic tool was mentioned by Fairmaire (1884) and Peyerimhoff (1923), and described in detailed by Bechyně (1948) for the definition of each of his species groupings. The vestiture in males is generally complete with the exception of the base of metatarsomere II and III in most species; however, in females an interesting variation between species exists.

The everted and inflated endophallus was first studied by Stockman (1966) for three species: *Timarcha maritima* Perris, 1855, *T. normanna* Pasquet, 1923 (both referring to the same figure) and *T. tenebricosa* (Fabricius, 1775). Recently, Petitpierre & Anichtchenko (2018) published an important study on the inflated endophallus of more than thirty nominal species. They found interesting variations among them, and also within the species *T. intermedia* Herrich-Schäffer, 1838.

Observations on the sclerites of the endophallus were made by Stockmann (1966), wherein he found no significant differences in some species of the *Timarcha goettingensis* group (*T. goettingensis*, *T. normanna*, *T. maritima*, *T. geniculata* (Germar, 1823)), yet did find a difference in *T. tenebricosa*. At the same time, Iablokoff-Khnzorian (1966) characterized the sclerites in *T. (Metallotimarcha) hummeli* and *T. tenebricosa*. Later, Petitpierre (1970) did a more extensive search on this character, figuring 15 species; he found important differences between most of them, but also remarked a high intraspecific variability. Petitpierre & Daccordi (2013) drew the sclerites of five species from Andalucía, namely *T. carmelenae* Petitpierre, 2013, *T. sagrensis* Kuntzen, 1911, *T. seidlitzi* Kraatz, 1879, *T. granadensis* Bechyně, 1948 and *T. marginicollis* (the figures of the first two are reversed). Lastly, Petitpierre (2019) showed the sclerites of 16 species.

This paper is an introductory one that will serve for our future studies revising the taxonomy of this genus. Thus, species analysed here are only scattered examples that show the results of the described techniques.

Material and methods

Abbreviations of the collections

MDVI = Mauro Daccordi collection, Verona, Italy
MBLI = Maurizio Bollino collection, Lecce, Italy
BVMS = G. Bastazo & J. M.Vela collection, Málaga, Spain

Studied specimens and their geographical source

Timarcha (Americanotimarcha) intricata Haldeman, 1853: U.S.A., Washington State, 1 ♂ (MDVI); U.S.A., Oregon, East Fork, Dairy Creek, 1 ♂ (MDVI).
T. (Metallotimarcha) metallica (Laicharting, 1781): Croatia, Dundovici, 1 ♂ (MDVI).
T. (s. str.) *balearica* Gory, 1833: Spain, Mallorca, 1 ♀ (BVMS), 2 ♂♂ (MDVI).
T. (s. str.) *goettingensis* (Linnaeus, 1758): France, Versailles, 1 ♀ (BVMS); Germany, Bad Frankenhausen, 2 ♂♂ (MDVI, MBLI); Austria, nr. Wien, 1 ♂ (MDVI).
T. (s. str.) *granadensis*: Spain, Granada, Puebla de Don Fadrique, 1 ♂ (MBLI).
T. (s. str.) *hispanica* Herrich-Schäffer, 1838: Spain, Madrid, Escorial, 1 ♂ (MDVI).
T. (s. str.) *intermedia* Herrich-Schäffer, 1838: Spain, Granada, Motril, 1 ♀ (BVMS); Spain, Granada, Castell de Ferro, 1 ♂ (MDVI); Spain, Almería, Níjar, 1 ♂ (MBLI).
T. (s. str.) *lusitanica* (Fabricius, 1781): Portugal, Oeiras, 1 ♀ (BVMS).
T. (s. str.) *maroccana* Weise, 1882: Morocco, Azerzou, 1 ♀ (BVMS); Morocco, Middle Atlas, Sidi Ali, 1 ♂ (MDVI).
T. (s. str.) *marginicollis* Rosenhauer, 1856: Spain, Granada, 1 ♂ (MBLI).
T. (s. str.) *nicaeensis* Villa & Villa, 1835: Italy, Alpi Liguri, Margheria dei Boschi, 1 ♀ (BVMS).
T. (s. str.) *pimelioides* Herrich-Schäffer, 1838: Italy, Sicily, Portella Misilbesi, 1 ♀ (BVMS).
T. (s. str.) *prujai* Kocher, 1963: Morocco, Bab Berret, 1 ♀ (BVMS).
T. (s. str.) *rugosa* (Linnaeus, 1767): Algeria, Biskra, 1 ♀ (BVMS); Algeria, Batna, 1 ♂ (MDVI).
T. (s. str.) *scabripennis* Fairmaire, 1868: Morocco, Souk-el-Khemis des Anjra, 1 ♀ (BVMS).
T. (s. str.) *strangulata* Fairmaire, 1861: Spain, Pyrenees, 1 ♂ (MBLI).

T. (s. str.) tenebricosa (Fabricius, 1775): France, Bretagne, Morlaix, 1 ♀ (BVMS); England, Launceston, 1 ♂ (MDVI); England, Cambridgeshire, 1 ♂ (MDVI).

Preparation and examination of the tarsi

Specimens were softened by boiling in hot water. Tarsi of females of selected species were separated, cleaned and mounted face up on a card. The tarsal vestiture of the female was categorized as complete or incomplete. The vestiture of the tarsus is incomplete when it has a glabrous line along the middle. In the different species, we can observe variation in the glabrous line: some lack a medial line (complete vestiture), some have a line along the entire length of the tarsomere, and others have a partial glabrous line. The vestiture of ventral part of the tarsomeres is herein described by means of a formula of three groups of three digits. The first group corresponds to the protarsomeres I, II, III, separated by a semicolon from the second group (mesotarsomeres I, II, III), which is separated by a semicolon from the third group (metatarsomeres I, II, III). Each number runs from 0 (complete vestiture, with absence of a medial hairless line) to 1 (a complete hairless line); the intermediate state is expressed by a fraction (>0 and <1) meaning that the medial hairless line is present in only a part of the length of the tarsomere, starting from the base. In some cases, the vestiture of the studied tarsomere was variable; in those occasions, the range of the variation is indicated.

Preparation and examination of the sclerites of the endophallus

The correct preparation of the sclerites is a non-conservative technique with respect to the endophallus membranes. The dissection and preparation was achieved by the following steps:

a. Softening of the specimen was achieved by boiling it for approximately 1–2 minutes in ±30 ml of water with a drop of acetic acid; boiling times depend on the size, dryness and preservation of the specimen.
b. Lift the elytra with the help of a micro-scalpel and forceps. Tear the lateral membrane of the abdomen to extract the aedeagus. The elytra can rarely be opened (in *Americanotimarcha* and *Metallotimarcha*) and the abdomen should be cut dorsally.
c. Soak the aedeagus in KOH 10% for 10–20 minutes and clean the tissues. Remove the tegmen.
d. Cut the penis from the base to the middle by the ventral side with the tip of a pair of fine forceps, Dumont nº 5 (or similar).
e. Take the base of the sclerites, visible by the basal foramen of the penis, with the forceps and softly pull them off.
f. Put the sclerites in pure acetic acid for 2–3 minutes and clean off the tissues carefully.
g. Next, place it in absolute alcohol for 2–3 minutes.
h. Glue it onto a transparent mounting card with DMHF resin (Dimethyl hydantoin formaldehyde), soluble in water (Steedman 1958; Liberti 2005), or Euparal, soluble in ethyl acetate or Euparal essence. Sclerites are usually glued laterally to better see the flagellum and the phanera; however, in the *T. rugosa* group it is advisable to glue them in dorso-ventral position due to the great separation between the wings of the phanera.

Preparation and examination of the endophallus

The eversion and inflation of the endophallus, with minimal variations, follows the Berti-Vachon technique (Bontems 2013) as indicated in the following steps.

a. Soften the specimen by boiling for 1–2 minutes in ±30 ml of water with a drop of acetic acid.
b. Open the lateral membrane of the abdomen to extract the aedeagus.
c. Put the aedeagus with the ring tegmen *in situ* in KOH 10%. Bring the liquid to a boil and allow it to cool. Keeping the tegmen *in situ* will later help to better seal the aedeagus on the needle shaft.

d. Place the aedeagus in pure acetic acid for two minutes to stop the KOH reaction, then rinse in water.
e. Screw a blunt end needle on a syringe with a Luer Lock tip. The size of the needle depends on the size of the sample. To evert *Timarcha*, we habitually used 26–30G needles, but occasionally thinner needles (32–34G) may be necessary.
f. Under a stereoscope, introduce the apex of the needle in the base of the penis, pushing it gently inside to at least two thirds of the total length. To make the operation easier use a needle with a slightly curved tip to follow the natural curvature of the penis.
g. To hold the piece, we use a common cotton thread in which we make a slip knot. The knot is tightened around the aedeagus (including the tegmen) to lock it on the needle; the loop of the loose end is left free to allow the knot to be loosen easily afterwards.
h. With the syringe full of air, under the stereoscope, push the piston gently until the complete eversion of endophallus. If a complete eversion cannot be achieved, it can be attempted using a syringe filled with demineralized water to exert a higher yet non-elastic pressure on the membranes. Demineralized water will reduce the risk of clogging the lumen of thinner needles. Once the eversion is obtained, the injected liquid is aspirated and the syringe removed. A syringe filled with air is reused; air is introduced until the membranes are fully tensioned.
i. Maintaining the pressure, dry the piece with a hair dryer for 20–30 seconds. To be sure that the piece is dry, remove the pressure. If the membrane is not dry, reapply pressure to the piston and dry further.
j. To remove the aedeagus from the needle, loosen the slip knot by pulling the head of the loop. Next, with a thin brush, wet the portion of aedeagus that is partly attached to the shaft of the needle and gently extract the piece.
k. Glue the sample with everted endophallus on the apex of a mounting card.

Results

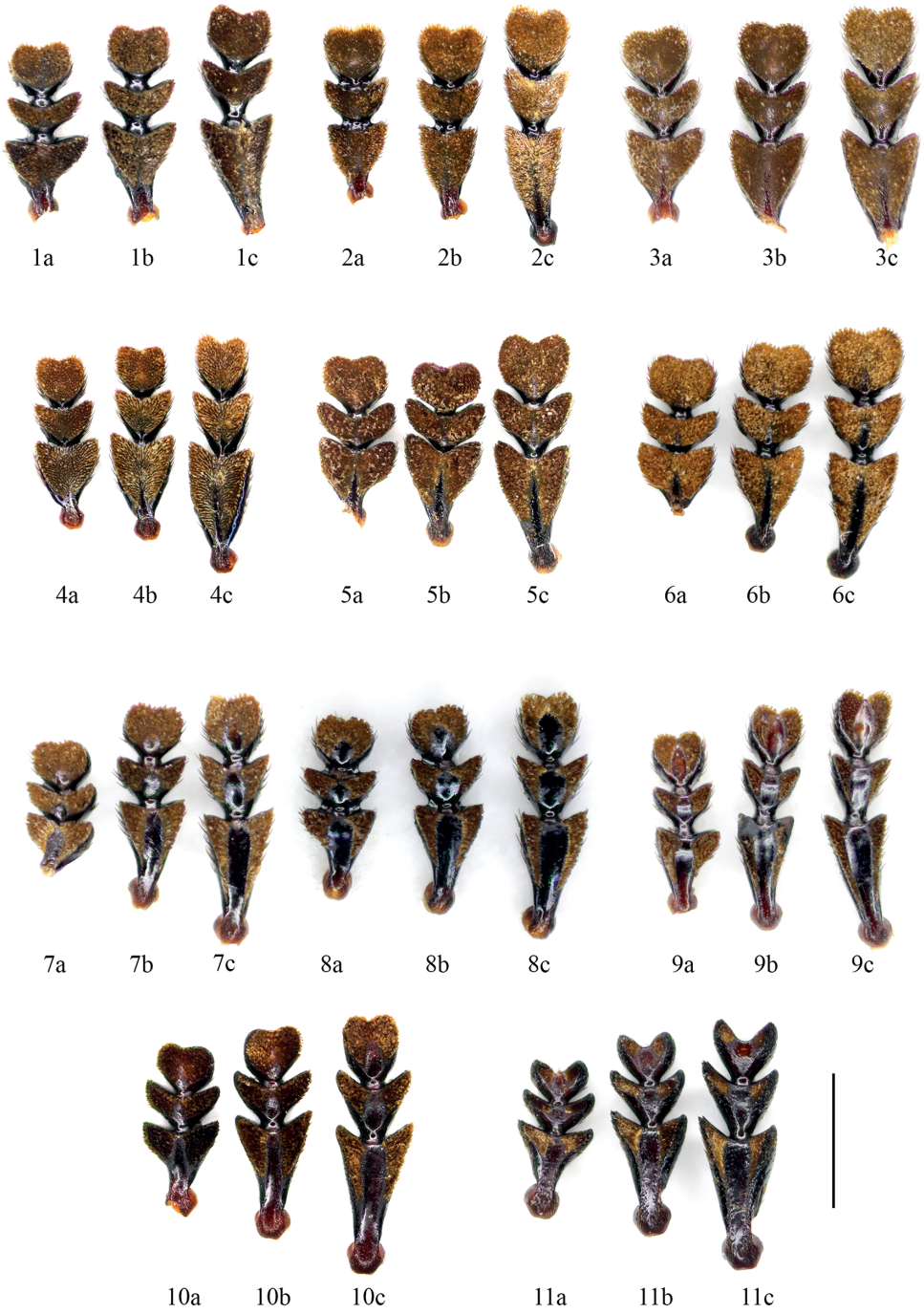
Class Hexapoda Latreille, 1825
Order Coleoptera Linnaeus, 1758
Suborder Polyphaga Emery, 1886
Superfamily Chrysomeloidea Latreille, 1802
Family Chrysomelidae Latreille, 1802
Subfamily Chrysomelinae Latreille, 1802
Tribe Timarchini Motschulsky, 1860

Genus ***Timarcha*** Samouelle, 1819

The female tarsal vestiture

Complete vestiture in females (0,0,0; 0,0,0; 0,0,0) were found in *Americanotimarcha* and *Metallotimarcha* species. Moreover, the species found in *Timarcha* s.str. with nearly complete vestiture were *T. maroccana* (0–¼,0,0; 0–¼,0,0; 0–¼,0,0) (Fig. 1) and *T. scabripennis* (0,0,0; 0–⅓,0,0; 0–⅓,0,0) (Fig. 2), whereas in the close *T. prujai* (Fig. 3) all the tarsomeres had an incomplete, narrow medial line (½–⅓,⅓–¾,⅓; ¾,¾,⅓–½; ¾,½–¾,⅓–½). *Timarcha balearica* (Fig. 4) showed a visible rather incomplete medial glabrous line in the pro and mesotarsomeres I and in metatarsomeres I and II (¼,0,0; ½–⅔,0,0; ⅔–1,½–¾,0).

On the other hand, *Timarcha rugosa* (Fig. 11) has a fully glabrous medial line in females (1,1,1; 1,1,1; 1,1,1). An almost fully glabrous medial line was found (1,1,¾–1; 1,1,¾–1; 1,1,1) in *T. intermedia* (Fig. 9), *T. carmelenae* Petitpierre, 2013 and *T. lugens* Rosenhauer, 1856. In *T. lusitanica* (Fig. 7) the line formula is (1,0–1,0–½; 1,½–1,½; 1,1,¼–¾). Greater variability was found in *T. goettingensis* (Fig. 8), with (1,1,⅓–¾; 1,1,⅓–¾; 1,⅘–1,½–¾), *T. pimelioides* with (⅓–1,0–1,0–¾; ⅓–1,¼–¾,¼–½; 1,¾–1,½–¾) (Fig. 10), and *T. tenebricosa* with (0–¾,0,0–¼; ¼–½,0–¼,0–¼; ½–1,¼–¾,¼) (Fig. 5). The



Figs 1–11. Female tarsomeres I–III in ventral view. **1**. *Timarcha maroccana* (Morocco, Azerzou). **2**. *T. scabripennis* (Morocco, Souk-el-Khemis des Anjra). **3**. *T. prujai* (Morocco, Bab Berret). **4**. *T. balearica* (Spain, Mallorca). **5**. *T. tenebricosa* (France, Bretagne, Morlaix). **6**. *T. nicaeensis* (Italy, Alpi Liguri, Margheria dei Boschi). **7**. *T. lusitanica* (Portugal, Oeiras). **8**. *T. goettingensis* (France, Versailles). **9**. *T. intermedia* (Spain, Granada, Motril). **10**. *T. pimelioides* (Italy, Sicily, Portella Misilbesi). **11**. *T. rugosa* (Algeria, Biskra). Abbreviations: a = protarsomeres; b = mesotarsomeres; c = metatarsomeres. Scale bar = 2.0 mm.

last one was similar to that of *T. nicaeensis* (½–⅔,0–¼,0; ½–¾,0–¼,0; ⅔–¾,0–½,0) (Fig. 6). Notably the morphology of tarsomeres III in *T. rugosa* is strongly emarginated (Fig. 11). Thus the morphology of tarsomeres may be useful to separate closely related species. For example, in *T. scabripennis* the tarsomeres I and II are not very dilated to the apex (Fig. 2), in *T. prujai* they are slightly dilated (Fig. 3) and in *T. maroccana* they are strongly widened (Fig. 1).

The sclerites of endophallus

The sclerites have different patterns in the three subgenera. The species of the subgenus *Timarcha* have sclerites of the endophallus which generally consist of a long, fine tube called the flagellum ("tige" for Iablokoff-Khnzorian 1966), that includes the ductus. The flagellum is a long, narrow plate curved in on itself which forms a tube that directs the sperm (see Fig. 23). The base of the flagellum is joined to a sclerotized piece named the phanera (Stockman 1966; manubrium for Iablokoff-Khnzorian 1966; Petitpierre 1970) which is attached to the dorso-apical part of the endophallus (see Petitpierre & Anichtchenko 2018, and Figs 25–29). The phanera is usually a paired structure, with two pieces in the shape of wings. In some species, the phanera is unpaired and reduced with a simple stick shape.

The presence of a phanera with two well-developed wings occurs in *Timarcha tenebricosa*, where the long flagellum is interestingly curved upwards and enlarged near the apex (Figs 12, 21). *Timarcha goettingensis* has a shorter flagellum and two less sclerotized wings (Fig. 13) which, if not carefully prepared under the microscope, can turn on themselves, giving a somewhat different appearance; the apex of flagellum has a small, thin digit-like appendix next to the apical pore (Fig. 22). In *T. rugosa* (Fig. 14) there is a central, straight flagellum, with two rather lineal and rigid wings well separated from the base, which are observed easier dorsally than laterally (Fig. 14).

An unpaired but well developed phanera is found in *Timarcha hispanica* (Fig. 15), whereas the phanera is quite short in *T. intermedia* (Fig. 16) and *T. balearica*; the latter however is very notable due to its coiling flagellum which is formed by the fusion of two symmetrical halves (Figs 17, 23).

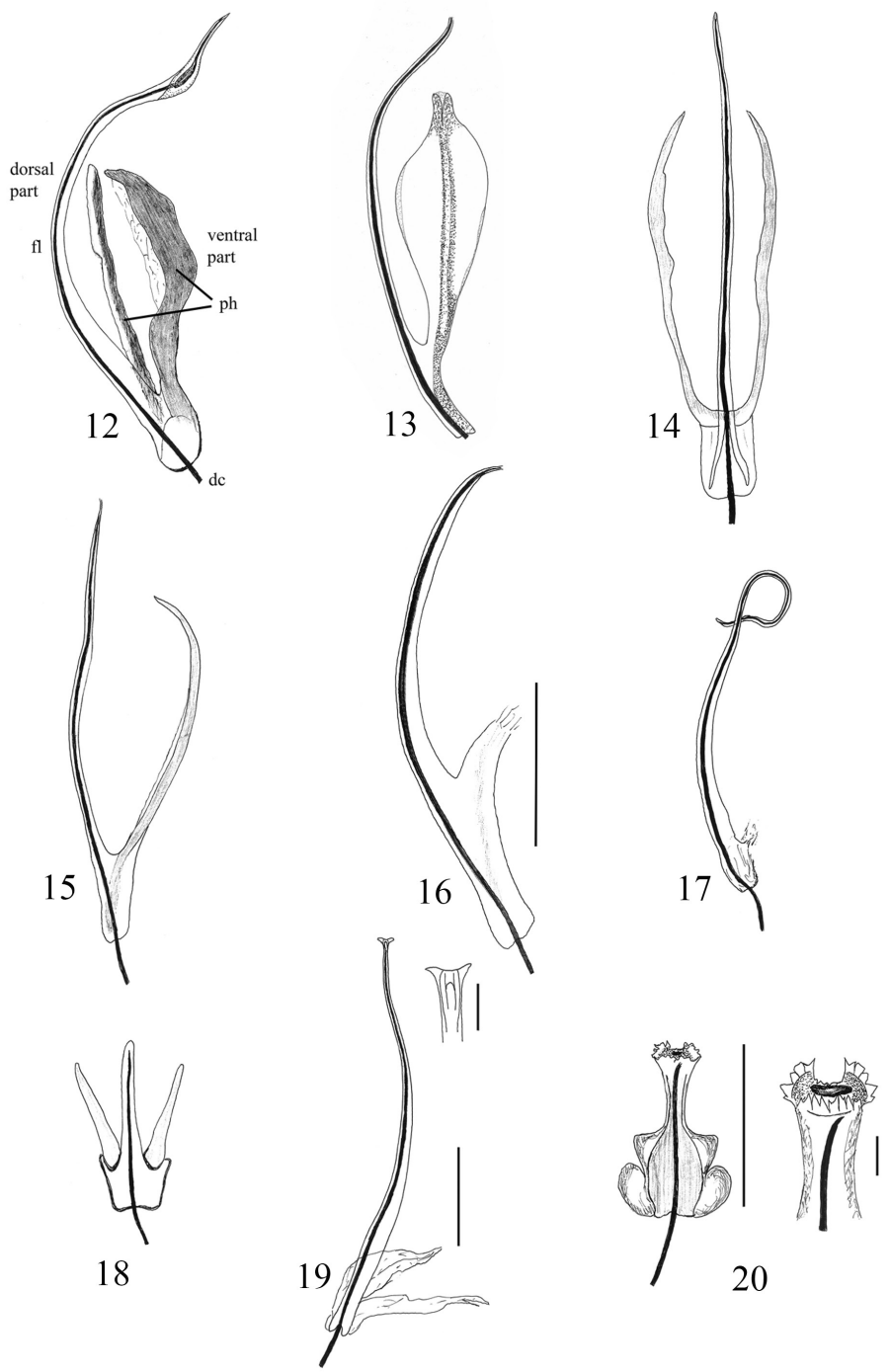
The endophallus sclerite of *Timarcha maroccana* (Fig. 18) is quite striking with a short structure, a paired phanera, which is lineal but well sclerotized, not winged, and a flagellum only slightly longer than the phanera.

On the other hand, in *Timarcha (Americanotimarcha) intricata* the flagellum is very elongated, hardly curved or almost straight, bifurcated at the base, decreasing in diameter and widening in two small divergent points at the apex. At the base there is a small, paired, very weakly sclerotized phanera (Fig. 19); close up it can be seen that the apex of flagellum has a digit-like appendix (Fig. 24)

In *Timarcha (Metallotimarcha) metallica*, the flagellum has a bifurcated base, is well chitinized, rigid, parallel sided, moderately elongated, ending in a slightly widened apex provided with a crown of denticles and an attached membrane. The phanera is paired, large, and incised at the base (Fig. 20). However, the sclerites of the endophallus are very different and characteristic in the distinct species of subgenus *Metallotimarcha* making it difficult to find a general pattern; although the base of flagellum always appears bifurcated, as occurs in *Americanotimarcha*.

The endophallus

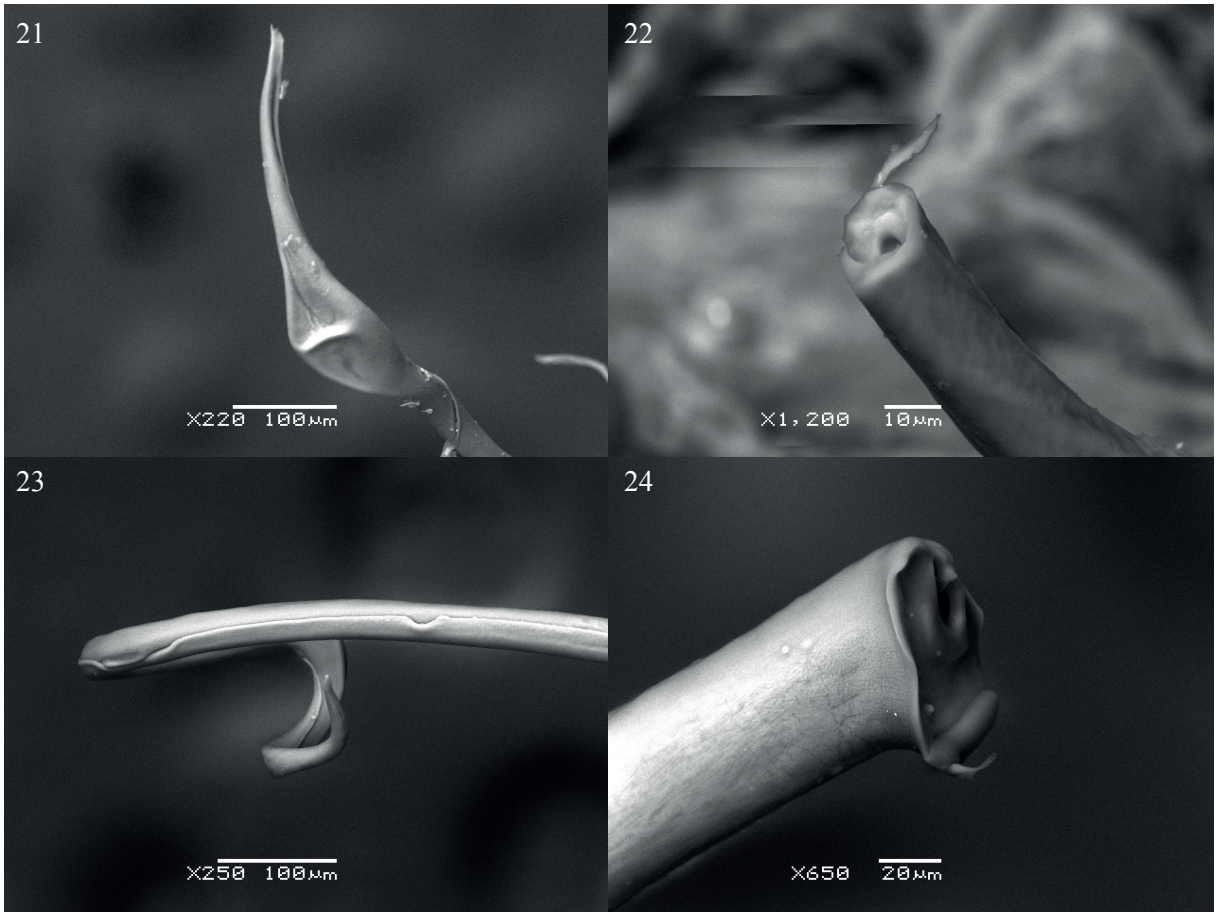
The endophallus (internal sac of penis) in *Timarcha* is simple, membranous, with bilateral symmetry, and is moderately variable between the species. Basically, it consists of three main lobes, in dorsal and/or ventral position: basal, medial and distal. The basal and medial lobes may have paired protuberances; while in the majority of the species evaluated, the distal one is unpaired. Lastly, the distal lobe can end in a small apical diverticulum. Besides this, the endophallus supports the sclerites described above; the



Figs 12–20. Sclerites of the internal sac of aedeagus. **12**. *Timarcha tenebricosa* (Launceston, England; dorsolateral). **13**. *T. goettingensis* (Germany, Bad Frankenhausen; dorsolateral). **14**. *T. rugosa* (Algeria, Batna; dorsal). **15**. *T. hispanica* (Spain, Madrid, Escorial; lateral). **16**. *T. intermedia* (Spain, Granada, Castell de Ferro; lateral). **17**. *T. balearica* (Spain, Palma de Mallorca; lateral). **18**. *T. maroccana* (Morocco, Middle Atlas, Sidi Ali; dorsal). **19**. *T. (Americanotimarcha) intricata* (U.S.A., Washington State; lateral). **20**. *T. (Metallotimarcha) metallica* (Croatia, Dundovici; ventral). Abbreviations: fl = flagellum; dc = ductus; ph = phanera. Scale bars: 12–18 = 0.5 mm; 19–20: short scale = 0.05 mm, long scale = 0.5 mm.

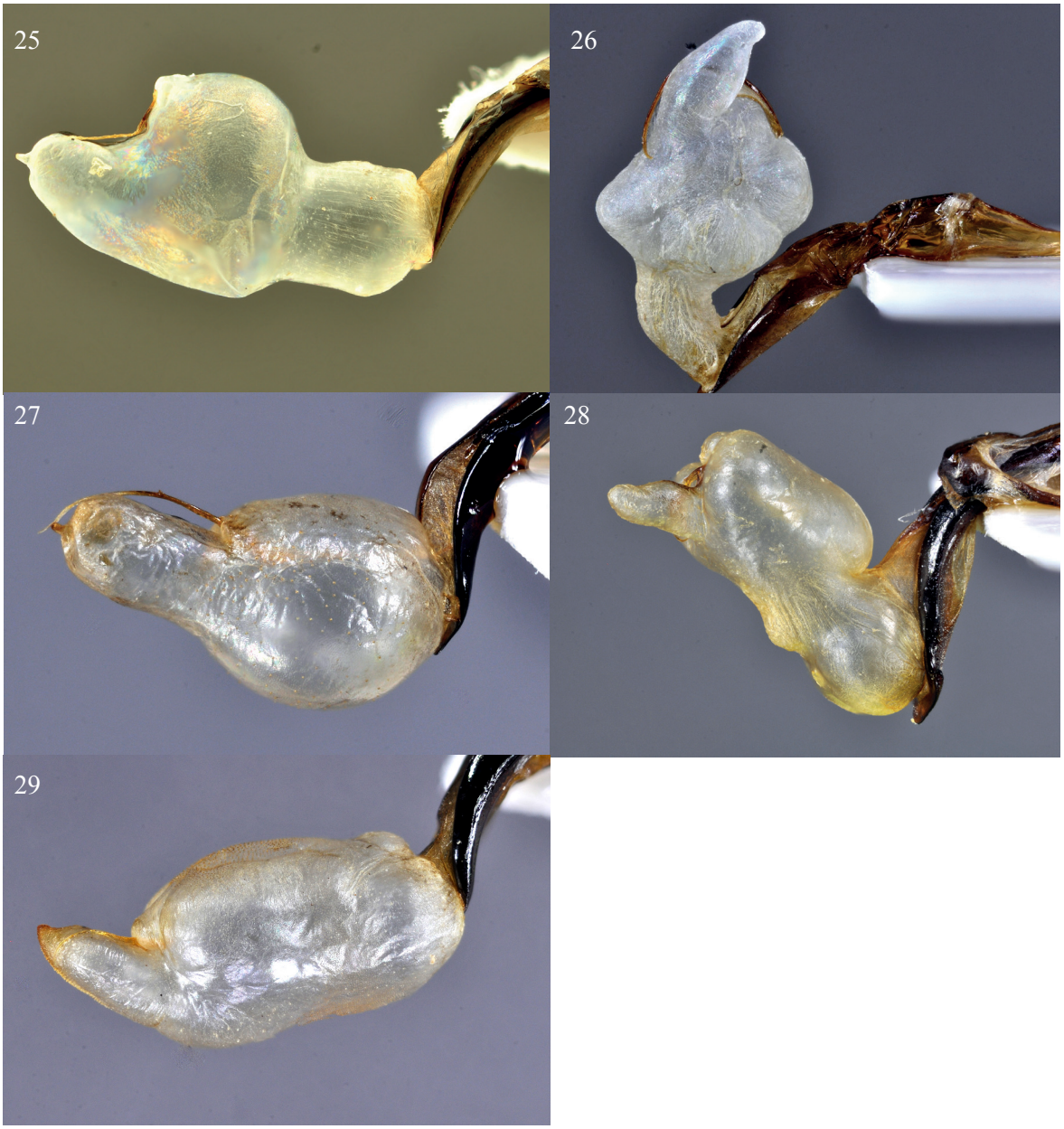
phanera is attached to the distal part of the dorsal protuberance of medial lobe. The endophallus is a fixation structure used to copulate, whereas the phanera serves to attach the flagellum which forms the sperm channel.

The endophallus is distinctive in different species. In *Timarcha goettingensis* (Fig. 25), there is a well-defined, long, basal lobe which opens to a widened medial lobe with a swollen dorsal protuberance and a weakly convex ventral protuberance. The distal lobe is developed and ends in a small apical diverticulum. On the other hand, *T. intermedia* (Fig. 26) has a narrow basal lobe, opening to a medial lobe with unpaired dorsal and paired ventral protuberances dilated with a long, tapering, distal lobe. In some species, like *T. granadensis*, there is not a basal lobe, but there is a swollen medial lobe and a distal lobe parallel sided, truncated at the apex, yet ending in a small distal diverticulum; interestingly, the medial lobe carries a number of tiny spines (Fig. 27; fig. 32 in Petitpierre & Anichtchenko 2018). *Timarcha marginicollis* (Fig. 28) has a rounded basal lobe, and a long, parallel-sided medial lobe, which has a developed dorsal proximal, two paired dorsal distal and ventral protuberances, and finally a finger-shaped distal lobe; no apical diverticulum was observed. In *T. strangulata* (Fig. 29), the basal lobe is not differentiated from the medial lobe, which has long dorsal and slight ventral protuberances; the distal lobe is thumb-shaped and slightly turned dorsally. A well-illustrated study of the interspecific variations is found in Petitpierre & Anichtchenko (2018).



Figs 21–24. SEM details of the apex of the flagellum. **21**. *Timarcha tenebricosa* (England, Cambridgeshire). **22**. *T. goettingensis* (Austria, Umgeb. Wien). **23**. *T. balearica* (Spain, Palma de Mallorca). **24**. *T. (Americanotimarcha) intricata* (U.S.A., Oregon, East Fork, Dairy Creek).

Theoretically, the ideal eversion technique should be simple, rapid, repeatable (even on the same sample, in case of initial failure), and applicable to any specimen (regardless of size); thus providing a permanent and easily studied sample. After trying various techniques, we have come to the conclusion that air filling (as suggested by Bontems 2013) is the one that better approximates the ideal technique. Techniques with other filling media, such as solution of gelatine powder, glycerine, water and Zinc Sulfate, which cause a white coloration to highlight the structures (Meurgues & Ledoux 1966), toothpaste (Janovska *et al.* 2013), K-Y gel (Van Damm 2014), absolute ethanol (Dang 1993; Hünefeld *et al.* 2013) or petroleum jelly (Yamasako & Ohbayashi 2011) always have some application limit. The toothpaste (or even the light-cured dental composite) and the gels are limited by viscosity, which prevents their



Figs 25–29. Air inflated endophallus. **25**. *Timarcha goettingensis* (Germany, Bad Frankenhausen). **26**. *T. intermedia* (Spain, Almería, Níjar). **27**. *T. granadensis* (Spain, Granada, Puebla de Don Fadrique). **28**. *T. marginicollis* (Spain, Granada). **29**. *T. strangulata* (Spain, Pyrenees). Not at the same scale.

use with very thin needles (Gauge < 30) and therefore with very small specimens; the absolute ethanol requires conservation of the piece in liquid, reducing the simplicity of study, and limiting the options for preservation together with the specimen. Furthermore, the problem of long-term preservation arises with fillers such as toothpaste or K-Y gel. Janovska *et al.* (2013) explained that some brands of toothpaste were well preserved after ten years, while in other cases after a few months the preparations were damaged by salt crystals; Van Damm (2014) does not provide information on the duration of preparations filled with K-Y gel.

In our opinion, air offers the following advantages:

1. Obviously, air flows through any needle, including the 36G needle (a needle with an external gauge of 0.127 mm and internal gauge of <0.08 mm), allowing, in theory, the eversion of even very small specimens like *Cryptocephalus* Geoffroy, 1762, *Pachybrachis* Chevrolat, 1837 and Alticini, for example.
2. The everted and dried piece can always be re-prepared by immersing it for a few minutes in hot water to rehydrate the tissues; this is especially true in cases where one realizes that complete eversion has not been achieved.
3. The endophallus' membranes are almost always transparent or semitransparent, allowing an easy study of the chitinized parts.
4. We know of *Timarcha* endophallus preparations, using only air, made by André Vachon and Nicole Berti more than 40 years ago and still in perfect condition (specimens studied by M. Daccordi kept in coll. Jean-Claude Bourdonné).
5. The everted and dried piece, once glued on a glue-board and pinned under the specimen, is unlikely to suffer damage, except by clumsy manipulation, which is always desirable to avoid.

In reality, the only limits to the air eversion technique are the manual skills of the operator and the availability of sufficiently thin equipment. Currently 34G needles are available without great difficulty, while 36G or 37G needles (the thinnest needles produced with current technologies) are difficult to find and very expensive.

Discussion

Taxonomy of *Timarcha* is undoubtedly difficult; it has been rather confounding for more than 250 years. Notwithstanding, there are structures of high diagnostic value, such as the endophallus and its sclerites (Petitpierre 1970, 2019; Petitpierre & Anitschenko 2018). Here a problem arises; if a dissection of the sclerites is performed, the endophallus is destroyed. Conversely, if the endophallus is everted with an opaque medium, sometimes the sclerites cannot be fully observed. However, the two characters are without doubt very useful to separate and identify all the *Timarcha* species. Sclerite dissection is obviously much easier and quicker than the eversion of the endophallus. Eversion of the endophallus may be difficult in the beginning, with a high percentage of failed attempts which will undoubtedly reduce with practice. But if, during the eversion process, the endophallus membrane is broken it is still always possible to prepare and preserve the sclerite.

The morphology of the tarsal vestiture in females is a good diagnostic characteristic in the most species, as illustrated, but not all. Mostly, it is a complementary character that can be useful when studying both sexes in a series of specimens of the same species.

Of course, there are other characters that should be considered for a correct species identification, since some of them are good diagnostic characteristics for some species; those being, for example, the shape of the aedeagus, shape of the pronotum, punctuation or shape of mesoventrite, even though they are highly variable in other species. By means of the use of all the studied characteristics, and the study of

the existing types, the taxonomy of *Timarcha* can finally be put in order. For many years two of us (M. Daccordi and J. M. Vela) have been and continue to systematically revise the majority of the types of the taxa at the species level, including by the dissection and study of the sclerites of nearly 850 specimens. It is noteworthy to comment that working on *Timarcha* taxonomy without examination of types could lead to erroneous taxonomic statements or classifications. After our preliminary work, the number of valid taxa at specific level in *Timarcha* is much smaller than had been supposed, with only about 60 valid species/subspecies worldwide (Daccordi & Vela, unpubl. data). So, the estimate of Warchałowski (2010) that 80% of the described species/subspecies in this genus should be considered as synonyms, is not far from reality.

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