



309

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Description of the full-grown larva and barcode of *Athripsodes taounate taounate* Dakki & Malicky 1980 (Trichoptera: Leptoceridae), an Iberic-Maghrebian endemic.

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Abstract

In this paper we describe the main morphological characteristics that distinguish the full-grown larva of *Athripsodes taounate taounate*, an Iberic-Maghrebian endemic. The conspecificity of the larva and adult was confirmed by DNA analysis. Morphological features that discriminate it from the described Iberian-Maghrebian species of *Athripsodes* are given.

Key words: caddisfly, morphology, DNA sequence, mtCOI, GenBank

Introduction

The genus Athripsodes Billberg 1820 is represented by about 33 species and subspecies in Europe and North Africa (Malicky 2005) of which 13 are distributed on the Iberian Peninsula (Coppa et al. 2022; González 2023; González & Martínez-Menéndez 2011; Martín et al. 2016), including A. albifrons (Linnaeus 1758), A. alentexanus Martín, González, Martínez 2016, A. aterrimus (Stephens 1836), A. bessae Malicky & Terra 1984, A. bilineatus bilineatus (Linnaeus 1758), A. braueri (A.-E. Pictet 1865), A. cuneorum (McLachlan 1884), A. curvispinus Coppa & González 2022 (in Coppa et al. 2022), A. inaequalis (McLachlan 1884), A. leucophaeus (Rambur 1842), A. taounate taounate Dakki & Malicky 1980 (also in Morocco), A. tavaresi (Navás 1916), and A. verai González & García de Jalón 1987; A. ygramul Malicky & Lounaci 1987 occurs only in Algeria. On the other hand, the larval taxonomy is little advanced in this genus and, on the Iberian Peninsula and in the Maghreb, the immature stages of only A. albifrons, A. aterrimus, A. bilineatus bilineatus, A. braueri, A. leucophaeus, and A. tavaresi are known (Waringer & Graf 1997, 2011; Vieira-Lanero 2000; Wallace et al. 2003; Forcellini et al. 2013). Iberian and Maghrebian larvae remain unknown for A. alentexanus, A. bessae, A. cuneorum, A. curvispinus, A. inaequalis, A. taounate taounate, A. verai, and A. ygramul. In the rest of Europe and North Africa, only the larvae of A. cinereus, A. commutatus, A. genei, A. longispinosus longispinosus, A. longispinosus paleochora, and A. bilineatus aegeus are known (Waringer & Graf 1997, 2011; Wallace et al. 2003; Waringer & Graf 2014; Waringer & Malicky 2019), with those of the 13 other species and subspecies still unknown.

The male of *A. taounate taounate* was described originally from specimens from Morocco and later found in southern Spain (González *et al.* 1990), Portugal (González *et al.* 1992), and more widely on the Iberian Peninsula (Martínez 2014). The species is distributed throughout the westernmost Mediterranean area. Variability observed in the species led to the distinction of subspecies such as *A. taounate algiricus* Botosaneanu 1989 from Algeria, now considered synonymous with *A. ygramul* (Malicky 2005), and *A. taounate siculus* Cianficconi 2001 for specimens from southeastern Sicily. The exhaustive analysis of a small collection of purported *A. taounate taounate* specimens from southern Portugal revealed that they belong to the recently described species *A. alentexanus*.

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In this paper we describe and illustrate the main morphological characteristics of the full-grown larva of *A*. *taounate taounate*, which allows us to differentiate it from the rest of the described Iberian-Maghrebian and European species. In addition, we provide the mtCOI barcodes of specimens from the southern part of the Iberian Peninsula.

Material and methods

Material examined. Five larvae and 28 adults (21 males, 4 females—22-vi-1995; 3 males—17-vi-2022) were collected from Benamahoma, El Bosque River (Guadalete Basin), Sierra de Grazalema Natural Park, 408 m a.s.l., UTM: 30S2740. [Other adults were examined from Río San Juan (Castillo de Locubín), 638 m a.s.l.: 18 males and 5 females (03-vii-2015), UTM: 30S4141; Vado del Genal (Pujerra), 515 m a.s.l.: 47 males and 32 females (23-vi-2012), UTM 30S3040.] All of the samples were taken by A. Ruiz-García using aerial and aquatic nets along a stream section.

The conspecificity of larvae and adults was confirmed by DNA analysis. In addition, species affiliation was enabled by the fact that both larvae and adults were collected at the same location, and that other potentially confusing species were missing (Waringer & Malicky 2019).

Collected material was fixed in 96% ethanol. Morphological characters were studied under a stereomicroscope (Nexius Zoom NZ. 1703-P) and photographed with a CMEX-10-PRO Digital Camera using Image Focus Alpha, and Helicon Focus software, when necessary. In the description, the chaetotaxy follows those of Williams & Wiggins (1981) and Wallace *et al.* (2003).

DNA analyses

In addition to morphological analysis, DNA was extracted from approximately 0.1 g of each specimen, using a guanidine hydrochloride-silica-based DNA extraction method described at the DNA Barcoding 101.org webpage. Samples were amplified with DNA primers LCO1490 and HCO2198 (Folmer *et al.* 1994) under the following PCR conditions: Initial denaturation at 94°C for one minute, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 45 seconds. PCR products were purified by isopropanol-ammonium acetate precipitation at room temperature and sequenced at an external facility (STAB VIDA, https://www.stabvida.com). Sequences were edited using the program Chromas Lite, and consensus was obtained using SeqTrace software (Stucky 2012). Identification was performed using Boldsystems' resources that use Hidden Markov Model (HMM) profile of the mtCOI protein, followed by a linear search of the reference library (Sujeevan & Hebert 2007). Sequences were deposited in GenBank. Reference sequences for other species were retrieved from GenBank (see Table 1 for accession numbers). A distance matrix was obtained using MEGA X software (Kumar *et al.* 2018). After a multiple ClustalW alignment, genetic distances were scored using the Maximum Composite Likelihood model (Tamura *et al.* 2004).

Description of the final (fifth) instar larva of *Athripsodes taounate taounate* **Dakki & Malicky, 1980** (Figs 1–18)

Biometry. Body length of final instar larva 10.5-12.0 mm, head width 0.78-0.89 mm (n = 5).

Head. Head capsule elongate and hypognathous; yellowish with pair of dark bands overlying and lateral of frontoclypeal sutures, converging posteriorly and reaching coronal suture; muscle attachment spots dark brown and round, with well-defined outlines. arranged in dark bands and posterolaterally on parietalia (Fig. 1). Head capsule with complete set of primary setae. Frontoclypeus narrow and triangular with constriction at eye level; three pairs of muscle attachment spots on anterior third and five on posterior two-thirds. Subocular ecdysial line (Fig. 2, black arrow) on each side running from frontoclypeal suture anterior of eye downward, then below eye to occipital foramen, making pale gap in dark margin of occipital foramen. Antennae uncharacteristically small for family, originating from socket-like ridge at anterior edge of parietalia (Fig. 2, red arrow), each bearing apical seta longer than basal sclerite of antenna. Labrum yellowish, subquadrangular with round apicolateral corners; anterior margin broadly concave; four pairs of setae inserted on dorsal surface. Ventral apotome chestnut brown, elongate,

subtriangular; tapered to mid-length and nearly parallel-sided in posterior half; posterior apex with arrowheadshaped tip; adjacent parts of ventral parietalia very dark (Fig. 4). Dark brown mandibles twice as long as wide, each with two setae near lateral base, proximal seta shorter than distal seta, and with two cutting edges, one dorsal and one ventral (Fig. 3).

TABLE 1. Intra- and interspecific uncorrected pairwise distances (p) in % of nucleotide differences of the mitochondrial cytochrome oxidase I (mtCOI) gene recorded for the sequenced *Athripsodes tauonate taounate* specimens of the Iberian Peninsula and other, previously sequenced European specimens, with GenBank and Boldsystems accession numbers. * = specimens sequenced in this study; M = male; L = larva.

Species	1	2	3	4	5	6	7	8	9	10	Acc. number
1. Athripsodes braueri											HM395952.1
2. Athripsodes cinereus	6.899										MW459908.1
3. Athripsodes aterrimus	14.715	14.897									HM395761.1
4. Athripsodes bilineatus	13.897	15.065	15.843								KX296553.1
5. Athripsodes albifrons	16.991	15.284	16.115	11.362							KX104615.1
6. Athripsodes commutatus	15.352	14.980	15.580	11.632	9.856						UMNEB118-08
7. Athripsodes alentexanus	0.411	6.893	14.537	14.213	17.144	15.336					IBITR437-20
8. Athripsodes tavaresi	14.198	13.652	18.529	15.649	16.050	17.722	14.541				IBITR268-20
9. Athripsodes inaequalis	10.932	11.377	14.545	14.386	16.484	16.111	10.924	12.162			IBITR137-20
10. Athripsodes taounate*									11.682		OR669954
(L)	7.351	7.763	14.688	15.041	16.069	16.267	7.646	14.441			
11. Athripsodes taounate*	7.351	7.763	14.688	15.041	16.069	16.267	7.646	14.441	11.682	0.000	OR669953
(M)											

Thorax. Pronotum with pair of yellowish sclerites, each sclerite with dark brown muscle attachment spots in posterior half and prominent spot in middle; posterior margin black; about 27–30 setae restricted to anterior half of surface; anterior margin with row of 50–60 pale setae of unequal length (Figs 5, 6); prosternal horn lacking (Figs 7, 13). Mesonotum with pair of yellowish sclerites, each with dark brown muscle attachment spots anteriorly and with black and diagonal mesonotal bar posterolaterally; each mesonotal sclerite with 30–35 setae concentrated on anterior half (Figs 6, 12); mesosternum without any setae (Figs 7, 13). Metanotum unsclerotized, with pair of anterolateral groups of sa3 setae each with 17–21 setae and posteromedian quartet of sa1 setae of almost equal length (Figs 6, 12, red arrows); metasternum with 1 pair of submedian setae (Fig. 7, red arrows).

Legs yellowish brown, fore- and mid- and hind legs successively longer with all tarsi curved and slender but never hooked; coxa, femur and tibia of each foreleg robust and broader than those of other legs (Figs 8, 9); each foretrochantin elongate with single black seta at distal margin (Fig 5, red arrow); ventral edge of each foretibia with two-three pale setae in addition to single thick spine at distal end (Fig. 11, black arrows). Trochanters of midand hind legs each divided into short proximal and longer distal sections (Fig. 8, black arrows). Hind femora each divided into short proximal section and longer distal section. Anterior faces of hind trochanters and femora without many short dagger-shaped setae (Fig. 10); numerous setae on coxae, trochanters, and femora of hind legs but long setal fringes for swimming lacking.

Abdomen. Membranous areas nearly white, sclerotized areas brown. First abdominal segment with two lateral and one dorsal protuberance (variably deflated, Figs 13 with black arrow; 16 with red arrow), lateral protuberances each with one pair of long black setae anteriorly (Fig. 16, red arrow) and numerous very tiny spines anteriorly on distinctive, very dark bar, its anterior end downward-curving about 90° and with seta at end (Figs 13, black arrow, 16, red arrow), its posterior end horizontal. Lateral fringe present on each side of segments III–VII, consisting of very short, pale hairs; lateral fringe on each side of segment II represented by two short black setae and on segment VIII by row of very short lateral tubercles (Fig. 18, black arrows; Wiggins 1996). Abdominal tergum VIII with four posterodorsal setae, submesal pair long (Figs 14 & 18, red arrows) and sublateral pair very short (Fig. 18, red arrow). Abdominal tergite IX well sclerotized and pentagonal, with ten setae on posterior margin, four pairs (1st, 2nd, 4th, 5th pairs) long and one pair (3rd pair) shorter (Fig. 14); one posterolateral seta present on each side of abdominal dorsum IX (Fig. 14, black arrows). Each lateral sclerite of last abdominal segment with five long setae and four or five short setae; anal region without rows of spines and tooth-edged plates; anal claws each with accessory hook (Fig. 15).

Tufted gills each with nine-thirteen filaments, except gills on each side of abdominal segment I with one anterior ventrolateral filament and three anterior subventral filaments (Fig. 13, red arrows); positions of gill tufts in abdominal segments I–III shown in Table 3 and Figs 12, 13, 16.

Case. Larval case 13–14 mm long (n = 5), curved, tapered (width at anterior opening 2.1–2.5 mm and at posterior opening 1.1-1.5 mm), composed of mineral grains of different sizes that give it an irregular texture (Fig. 17). Posterior opening closed by silken membrane with almost circular central foramen.

DNA analyses. The analysis of the mtCOI barcode region of one male of *A. taounate taounate* from Benamahoma (Spain) (GenBank accession number: OR669953) and one previously unknown larva collected in the same locality (GenBank accession number: OR669954) showed a genetic distance of 0.00% (Table 1). These values fit well within the intraspecific variability of mtCOI usually observed in caddisflies (Pauls *et al.* 2009, 2010; Previšić *et al.* 2009, 2014). Moreover, the uncorrected p-distances based on the mtCOI gene of these two individuals and other previously sequenced European specimens are in line with interspecific distances commonly reported in Leptoceridae (Kučinić *et al.* 2020; Ruiz-García *et al.* 2022).

Discussion

Characters for morphological discrimination of the final (fifth) instar larva of *Athripsodes taounate taounate* from those known for other known European *Athripsodes* spp., including species not recorded from the Iberian Peninsula and the Maghreb, are provided in Table 2.

TABLE 2. Diagnostic matrix for identification of the known larvae of European *Athripsodes* species and subspecies. Adapted from synoptic keys of Waringer & Graf (2014) and Waringer & Malicky (2019).

Species/character	Dorsal head surface	Hind trochanters and	Foretibia each with	Colour of	Dorsal	
	with clearly defined,	femora each with	ventral edge setae ²	submentum	parietalia with	
	dark, roundish	many short, dagger-	in addition to distal	(except	pair of dark	
	muscle attachment	shaped setae on	spine,	anterior	bands beside	
	spots	anterior face		margin)	frontoclypeal	
					sutures	
A. albifrons	no	no	no	dark	yes	
A. aterrimus	yes	yes	no	pale	yes	
A. bilineatus	yes	no	no	dark	yes	
bilineatus						
A. bilineatus aegeus	no	no	no	dark	no	
A. braueri	yes	no	yes (4)	pale	no	
A. cinereus	yes	no	yes (2)	dark	no	
A. commutatus	no	no	no	pale	no	
A. genei	no	no	yes	dark	no	
A. leucophaeus	no	no	yes (1)	pale	no	
A. longispinosus	no	no	yes (1)	dark	no	
longispinosus ¹						
A. longispinosus	no	no	yes (2)	dark	no	
paleochora ¹						
A. taounate	yes	no	yes (2–3)	dark	yes	
taounate						
A. tavaresi	no	no	no	pale	yes ³	

¹ In *A. longispinosus longispinosus*, the posterior apex of the ventral apotome is blunt, in *A. longispinosus paleochora* it is acute (Waringer & Malicky 2019).

² Number of additional edge setae of each foretibia in brackets ().

³ Vieira-Lanero (2000) distinguished a dark band on each parietal parallel to the frontoclypeal suture of *A. tavaresi*, but in his figure 72h such bands are not obvious and are similar in colour to the adjacent areas.

	Segment I	Segment II		Segment III		
Subdorsal		11–13	11–13	11–13	11–13	
Dorsolateral		11–13	11–13	11–13		
Pleural		9–10			Lateral line	
Ventrolateral	1	11–13	11–13	11–13	11–13	
Subventral	3	11–13	11–13	11–13		

TABLE 3.	Diagram of gil	l tufts and the	ir anterior	and posterior	positions	on left	side of	abdominal	segments	I–III (of
Athripsode	s tauonate tauon	nate. Numbers	denote au	antity of filam	ents per tu	ıft.					

DNA analysis to establish the conspecificity of larva and adults of Athripsodes taounate taounate

The molecular data enable confident association of the larva and the adult of *A. taounate taounate*. Furthermore, the association of larvae and adults is based not only on comparisons of sequences from these specimens as Zhou (2007) and Zhou *et al.* (2007) recommended, but also by their co-occurrence at the same locality.

In addition, the genetic analyses agree with the morphological characteristics, corroborating the close phylogenetic relationship of *A. taounate* with *A. braueri*, *A. cinereus*, and *A. alentexanus*. Although Pauperio *et al.* (2023) concluded that DNA barcodes, based on mtCOI (interspecific uncorrected pairwise distance (p) = 0.411, Table 1), might not differentiate between *A. braueri* and *A. alentexanus*, we consider the latter provisionally as a valid species. In the paper originally describing *A. alentexanus*, Martín *et al.* (2016) cited a paratype from Benamahoma, but we have examined numerous males captured in this locality, and others (see material examined), and have not found any specimen with the characteristics of that latter species. The distinctions among Iberian-Maghrebian species *A. alentexanus*, *A. braueri*, and *A. taounate* require clarification, as also suggested by Neu *et al.* 2018. Any morphological distinctions between larvae of the subspecies of *A. taounate* also should be described.

Ecological Remarks

In the Sierra de Grazalema Natural Park, the larva of *A. taounate taounate* inhabits headwater streams and springs with calcareous freshwaters and even naturalized irrigation channels (Figs. 19, 20) flowing with high-quality waters and substrate composed mainly of sand, gravel, and larger stones.

Athripsodes taounate taounate shares this stream section with a characteristic larval community of caddisflies including Hydropsyche instabilis (Curtis 1834), Hydropsyche infernalis Schmid 1952, Silonella aurata (Hagen 1864), Rhyacophila munda Mclachlan 1862, Adicella reducta (McLachlan 1865), Micrasema moestum (Hagen 1868), Allogamus kampos Oláh & Ruiz-Garcia 2014 (in Oláh et al. 2014), Sericostoma vittatum Rambur 1842, Polycentropus kingi McLachlan 1881 and Calamoceras marsupus Brauer 1865.



FIGURES 1–8. Larva of *Athripsodes taounate taounate* Dakki & Malicky 1980: (1) head capsule, dorsal; (2) head capsule, right lateral; ecdysial line, black arrow; antennal apical seta, red arrow; (3) left mandible, ventral; (4) head capsule, ventral; (5) prothorax, left lateral; foretrochantin, red arrow; (6) head, thorax, left dorsolateral; metanotal setal areas *sa*2 and *sa*3, red arrows; (7) thorax, ventral; metasternal setae, red arrows; (8) left legs, posterior faces: dt: distal section of trochanter; pt: proximal section of trochanter; df: distal section of femora; pf: proximal section of femora. All figure scales: 1 mm, except (3) scale: 0.5 mm.



FIGURES 9–13. Larva of *Athripsodes taounate taounate* Dakki & Malicky 1980: (9) right legs, anterior faces; proximal (pf) and distal (df) hind femora; (10) left hind trochanter and femur, anterior faces; (11) left foreleg, anterior face; ventral edge of its tibia with 3 pale setae (black arrows) in addition to single spine at distal end; (12) meso- and metathorax and abdominal segments I–IV, right dorsolateral; single seta on each anterodorsal setal area *sa*1 of metanotum, red arrows; single seta on each anterodorsal setal area *sa*1 (black arrows) and dorsal protuberance (ovoid dashed line) of abdominal segment I; (13)) thorax and abdominal segments I–IV, ventral; left ventrolateral and subventral gills of abdominal segment I, red arrows; left lateral protuberance, black arrow. All figure scales: 1 mm, except (11) scale: 0.5 mm.



FIGURES 14–18. Larva of *Athripsodes taounate taounate* Dakki & Malicky 1980: (14) tip of abdomen, right dorsolateral; posterolateral setae on abdominal dorsum IX, black arrows; posterodorsal seta on abdominal segment VIII, red arrow; (15) tip of abdomen, ventral; (16) abdominal segments I–IV, right lateral; right lateral protuberance, red arrow, and setae of setal area *sa*1, black arrow, of abdominal segment I; right ventrolateral gill of abdominal segment I, yellow arrow; (17) larval cases, lateral; (18) abdominal segment VIII, right lateral; row of lateral tubercles, black arrows; posterodorsal seta on abdominal segment VIII, red arrow. All figures scale: 1 mm, except (18) scale: 0.5 mm.



FIGURE 19. Sierra de Grazalema Natural Park, Benamahoma, El Bosque River: Habitat of *Athripsodes taounate taounate taounate* Dakki & Malicky 1980.



FIGURE 20. Sierra de Grazalema Natural Park, Benamahoma, naturalized irrigation channel: Habitat of *Athripsodes taounate taounate* Dakki & Malicky 1980.

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