

***Onychogomphus cazuma* sp. nov. from Spain: Molecular and morphological evidence supports the discovery of a new European dragonfly species (Odonata: Gomphidae)**

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Abstract. *Onychogomphus cazuma* Barona, Cardo & Díaz sp. nov. is described from the mountainous inland area of Valencia in central-eastern Spain. The new species presents a combination of morphological characters that distinguishes it from all other species of the genus and can be readily identified by the morphology of the male appendages and the female vulvar scale, and by the shape of the median lobe of the prementum and the labial palps of the exuvia. Molecular analysis of two genetic markers, one nuclear and one mitochondrial (PRMT and COII), supports the full species rank for this new taxon, which is sister to the north-western African endemic *O. boudoti*. Despite its small known distribution and the vulnerability of its habitat, available data are still insufficient to place this new species into an IUCN Red List of Threatened Species category.

Further key words. Anisoptera, Iberia, Valencia, taxonomy, phylogeny

Introduction

Gomphidae, distributed all over the world except in Antarctica, is the third largest family of Odonata with 1 013 species described (SCHORR & PAULSON 2020). This species richness is heterogeneously distributed over the globe (DIJKSTRA & KALKMAN 2012); in the Western Palaearctic region, only 22 gomphid species are known, while in similar latitudes in North America,

gomphid diversity is considerably higher (DIJKSTRA & LEWINGTON 2006; KALKMAN et al. 2008; BOUDOT & KALKMAN 2015).

At present, 40 species are classified in the genus *Onychogomphus* Selys, 1854 (SCHORR & PAULSON 2020), distributed over the Palaearctic, Afrotropical and Oriental regions (DIJKSTRA & KALKMAN 2012; FERREIRA et al. 2014b). It is almost certain that this genus is polyphyletic and probably all tropical species should be moved to other genera (DIJKSTRA & KALKMAN 2012).

Three species of *Onychogomphus* have been reported so far from the Iberian Peninsula but none are endemic to this area. Their life-cycle, behaviour, larval and adult morphology, geographic distribution and ecological dynamics are relatively well known (FERRERAS-ROMERO et al. 1999; REHFELDT 2003; CANO VILLEGAS 2009; PRUNIER 2018; BOUDOT & KALKMAN 2015; VELASCO-VILLANUEVA et al. 2018).

Only one phylogenetic hypothesis including Western Palaearctic *Onychogomphus* species has been developed (FERREIRA et al. 2014b). In that study, a new lineage from Morocco was described based on molecular (COII and PRMT genetic markers) and morphological evidence: *Onychogomphus boudoti* Ferreira, 2014. The relationships of that new taxon were not fully resolved in the estimated phylogenies. However, it is not expected that *O. boudoti* is closely related to the Afrotropical fauna, since morphologically it is similar to the Western Palaearctic species.

In 2017, while carrying out a study on threatened odonates in the Escalona river basin, in the province of Valencia in Spain (BARONA 2017), individuals that differed morphologically from all other Western Palaearctic *Onychogomphus* were observed. In 2019, additional fieldwork in eastern areas of the Iberian Peninsula yielded males, females, larval stages and exuviae of these particular populations. The hypothesis that these individuals represented an undescribed taxon is tested in this study using morphological and molecular data, resulting in the description of a new species.

Material & methods

Data collection

Initial field trips were carried out in 2017, visiting 15 watercourses in the Escalona river basin (BARONA 2017), including rivers Cazuma, Fraile, Grande, and Barranc de la Manyana (Fig. 1).

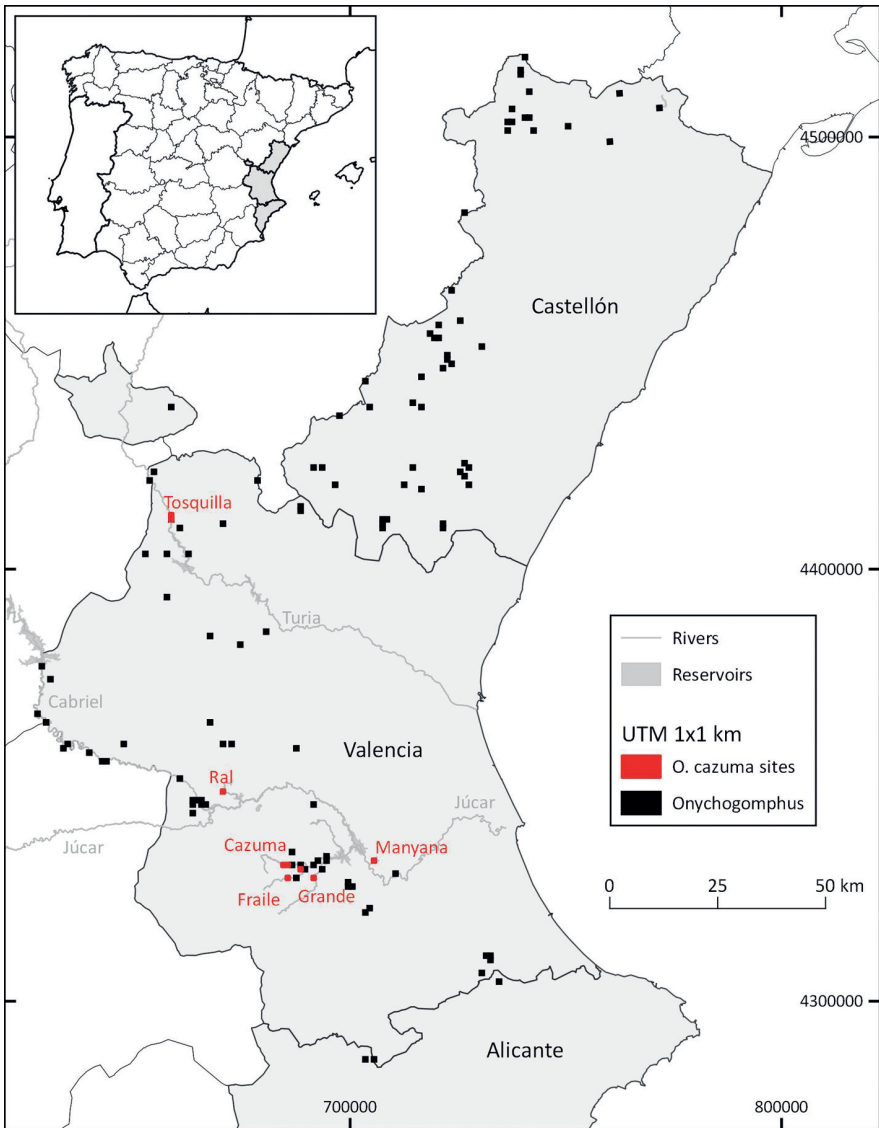


Figure 1. UTM 1 × 1 km squares with confirmed records of the genus *Onychogomphus* in the Comunidad Valenciana (comprising the provinces of Alicante, Valencia and Castellón) and known sites for *Onychogomphus cazuma* sp. nov.

We reviewed specimens in five entomological collections (collection acronyms are given in parentheses): Consejo Superior de Investigaciones Científicas, Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Spain (ICBIBE); Colección de Entomología del Área de Biología Animal del Departamento de Zoología y Antropología Física, Universidad de Murcia, Spain (UMCZ); Colección de entomología, Centro Iberoamericano de la Biodiversidad, Universidad de Alicante, Spain (CEUA); Museu de Ciències Naturals La Salle, Paterna, Valencia, Spain (CLS-MHLP).

In addition, we checked the following citizen science websites for *Onychogomphus* pictures from eastern Spain: <https://www.biodiversidadvirtual.org/>; <http://www.bdb.gva.es/es>; <https://observation.org/>. Finally, we contacted local photographers and amateur entomologists and reviewed our own photographic archives, gathering additional pictures of *Onychogomphus* individuals from the Comunidad Valenciana (Fig. 1).

As a result of the preliminary outcomes of the entomological collection and photographic files surveys, in 2019 we extended fieldwork to two additional sites (La Tosquilla, Rambla del Ral, Fig. 1) where specimens with the features under study were expected.

Measurements [mm] were taken using a digital calliper. Laboratory photographs were taken with a Leica M165C stereoscope coupled with a Leica DFC450 camera and processed with the program Leica Application Suite X. The map was created in QGIS v.3.0.1, using Universal Transverse Mercator (UTM) coordinate system.

DNA extraction and sequencing

Genomic DNA was extracted from a tissue sample of the thoracic muscle of the hind leg using the 'DNA Easy extraction Kit' (Qiagen®) following the manufacturer's protocol. Partial sequences of the nuclear arginine methyltransferase (PRMT) and mitochondrial cytochrome oxidase subunit II (COII) genes were amplified by polymerase chain reaction (PCR) using the following set of primers: ARG_F2/ARG_R3 (FERREIRA et al. 2014a) and COII-F-SF/COII-R-SF (FERREIRA et al. 2014b) respectively. These markers were selected based on their proven efficiency in previous phylogenetic

and phylogeographic studies of Odonata (BYBEE et al. 2008; FERREIRA et al. 2014a, 2014b; CARLE et al. 2015). PCRs were carried out in 25 µL of final reaction volumes containing: 17.5 µL of H₂O, 2.5 µL of a reaction buffer with MgCl₂, 1 µL of dNTP, 0.1 µL of MgCl₂, 0.5 µL of each primer, 0.2 µL of Taq polymerase (Nzytech[®]) and 2 µL of specimen DNA; reaction thermal cycling conditions were set as same as FERREIRA et al. (2014b). PCR products were sent to Macrogen[®] Spain to be sequenced in both directions by Sanger sequencing. Chromatograms were assembled and edited using Geneious[®] v11.0.5.

Phylogenetic analyses

Phylogenetic reconstruction was performed by assembling separate data sets for each gene (COII and PRMT) including sequences of *Onychogomphus* and related genera available in GenBank. Both matrices were aligned separately using the MAFFT algorithm through their online server (KATOH et al. 2017).

Bayesian analyses were performed with MrBayes version 3.2.6 (RONQUIST et al. 2012). Selection of the best model of substitution for each gene was calculated by setting the command *lset nst* to mixed. MrBayes analysis consisted of two simultaneous runs of 100 million generations each, sampling trees every 10 000 generations. Mixing and convergence among runs were evaluated by checking the average standard deviation of split frequencies and the EES values and Potential Scale Reduction Factor for each parameter. A majority consensus tree was reconstructed after discarding the first 20 000 sampled trees as burn-in. COII data set included 35 terminals and 732 pb while PRMT data set contained 21 terminals and 511 pb. Taxa included in this analysis, locality, GenBank accession and voucher numbers are provided in Table 1 and Appendix 1.

All analyses were run in the public resource CIPRES Science Gateway version 3.3 (MILLER et al. 2010).

Abbreviations

FW – Fore wing; HW – hind wing; Pt – Pterostigma; Ax – Antenodal cross-veins; Px – Postnodal cross-veins; S1–S10 – abdominal segments one to ten; m a.s.l. – metres above sea level.

Results

During 2017 and 2019, we collected the following material of the putative new species: nine adult specimens (6♂ 3♀), three larvae and ten exuviae. Additionally, we photographed 19 adult individuals (15♂ 4♀) and observed a further 19 adults (17♂ 2♀) (Table 1). In 2017, adult individuals with distinct morphology were observed in sympatry with *Onychogomphus uncatius* (Charpentier, 1840) and represented from 16.7% of total *Onychogomphus* individuals in the Cazuma river up to 100% in the Barranc de la Manyana (BARONA 2017).

Altogether, 184 *Onychogomphus* specimens were examined in five entomological collections (104 in MNCN; 67 in ICBIBE; 10 in UMCZ; 3 in CEUA; 0 in CLS-MHLP). Four dry-preserved specimens apparently related to the putative new species were found, three in ICBIBE (Table 1) and one in UMCZ. The former have the diagnostic features of the new species and have been designated as paratypes. A detailed morphological examination of the latter specimen, collected in the 1950s in Barinas, Murcia, revealed that it is a *chimaera*, as head, thorax and first segments of the abdomen belong to a specimen of *O. forcipatus* that is glued to the last four segments of an abdomen with the features of the new species. This specimen was excluded from the type series because we could not attribute it to a locality (the label can correspond to either or both specimens).

Type series of adult specimens and exuviae samples have been deposited in the following institutions: in the MNCN, male holotype and one female paratype dry-preserved and seven paratypes in alcohol, and in the ICBIBE three paratypes dry-preserved and two exuviae. In addition, larvae were reared by the authors.

We gathered pictures of 241 individuals of *Onychogomphus*, finding eight adults of the putative new species in 5 UTM 1 × 1 km squares in the Comunidad Valenciana (Table 1). From all data sources combined, we collected 304 records of *Onychogomphus* individuals from 122 UTM 1 × 1 km squares (Fig. 1). The new species has been found in nine UTM 1 × 1 km squares (7.4%) while *O. forcipatus* and *O. uncatius* have been found in 60 (49.2%) and 79 (64.8%) squares, respectively.

No additional sites were identified by reviewing 378 pictures of *Onychogomphus* from eastern Spain on citizen science platforms.

Table 1. Sites and records for *Onychogomphus cazuma* sp. nov. UTM 1 km – coordinates UTM datum ETRS89, 1 × 1 km grid; Elev. – elevation [m a.s.l.]; ph. – photographed; obs. – observed; coll. – collected; ten. – teneral; ex. – exuvia(e). GenBank accession numbers for specimens used for genetic comparison are added.

Site	UTM	Elev.	Records	Voucher nr	GenBank acc. number	
					COII	PRMT
Spain, Valencia, Antella, Barranc de la Manyana	30SYJ	100	09-vi-2017: 1♂ ph., 1♀ obs.; J. Barona 26-xii-2019: 3 larvae coll.; J. Barona & S. Teruel			
Spain, Valencia, Bicorp, Río Cazuma	30SXJ	435	14-vi-2017: 1♂ ph., 1♂ obs.; J. Barona 14-vii-2017: 4♂ ph., 1♂ coll.; J. Barona, N. Cardo & C. Díaz 23-vii-2017: 1♀ ph. A.M. García & F. Cervera 26-vii-2017: 1♂ ph.; T. Alcocer 08-viii-2017: 1♂ obs.; J. Barona 10-viii-2017: 1♀ ph.; J. Barona 11-viii-2017: 2♂ obs., 1♀ ph.; J. Barona 12-viii-2017: 1♂ ph.; J. Barona 12-vi-2018: 1♂ ten. ph., ex. coll.; M.J. Tarruella & S. Teruel 08-vi-2019: 1♂ ten. coll.; J. Barona, N. Cardo, C. Díaz & S. Teruel 16-vi-2019: 1♂ ten. ph., ex. coll.; S. Teruel 22-vi-2019: 4♂ 1♀ ex., coll.; S. Teruel	CAZ1/MNCN_ Ent255172	MT 415339	MT 415344
	30SXJ	8531				
	30SXJ	8431				

Site	UTM	Elev.	Records	Voucher nr	GenBank acc. number	
					COII	PRMT
			29-vi-2019: 1♀ ph.; Y. Maggioto			
			05-vii-2019: 2♂ ph., 1♂ coll.; J. Barona	CAZ3/MNCN_ Ent255167		MT 415346
			20-vii-2019: 1♂ ph., 1♂ coll., 1♀ coll.; J. Barona	CAZ5/MNCN_ Ent268518 CAZ6/MNCN_ Ent255168		
			21-vii-2019: 1♂ 1♀ ph., 1♀ coll.; J. Barona	CAZ7/MNCN_ Ent268519		
Spain, Valencia, Bicorp, Río Fraile	30SXJ	321	23-vii-2017: 2♂ obs.; J. Barona.			
	30SXJ	484	24-vii-2017: 1♀ ph.; J. Ordóñez & MJ. Tarruella			
Spain, Valencia, Quesa, Río Grande, Charcos de Quesa	30SXJ	281	14-vi-2016: 1♂ ph.; J. Pérez			
Spain, Valencia, Cortes de Pallás, Rambla del Ral	30SXJ	434	03-vii-2019: 1♂ ph.; T. Alcocer			
	7048		18-vii-2019: 1♂ obs., 1♂ coll., 1♀ coll.; N. Cardo & C. Díaz	RAL1/MNCN_ Ent255169 RAL2/MNCN_ Ent255170		
Spain, Valencia, Titaguas, La Tosquilla	30SXX	561	19-vi-2003: 1♂ coll.; P. González & J. Michelena	MUVHN_ENV 00018882		
	30SXX		24-vii-2003: 1♂ coll.; P. González & J. Michelena	MUVHN_ENV 00052471		
	5811		09-ix-2003: 1♂ coll.; J. Michelena	MUVHN_ENV 00050960		
			02-viii-2008: 1♀ ph.; S. Teruel			
			07-vii-2019: 10♂, 1♀ obs., 4♂ ph., 1♂ coll., 3 ♀ ex. coll. J. Barona & S. Teruel	TOS1/MNCN_ Ent255171		

***Onychogomphus* specimens used for genetic comparison**

Site	UTM	Elev.	Identification	Voucher number	GenBank accession number	
					COII	PRMT
Spain, Cuenca, Valdeganga, Río Júcar	30SWK6321	850	<i>O. forcipatus</i>	VAL1/MNCN_Ent255162	MT 415341	MT 415348
Spain, Cuenca, Valdeganga, Río Júcar	30SWK6321	850	<i>O. forcipatus</i>	VAL2/MNCN_Ent255163	MT 415342	MT 415349
Spain, Murcia, Abanilla, Río Chícamo	30SXH7434	280	<i>O. forcipatus</i>	CHI1/MNCN_Ent255161		MT 415350
Spain, Cuenca, Uña, Río Júcar	30TWK8652	1 120	<i>O. uncatu</i> s	UNA1/MNCN_Ent255166	MT 415343	MT 415347

Phylogenetic reconstruction

The topologies of the mitochondrial and the nuclear phylograms were totally congruent with each other (Fig. 2). Phylogenetic relationships for the taxa analysed coincided with those previously proposed by FERREIRA et al. (2014b). Our sequences from specimens found at the Valdeganga and Chícamo rivers were recovered within the *O. forcipatus* clade, while the sample from the Júcar river grouped with the *O. uncatu*s clade. However, sequences of specimens from the Cazuma river formed a separate group, sister to the Moroccan *O. boudoti* (PP = 1).

Molecular evidence indicates that the specimens from the Cazuma river represent a well-differentiated evolutionary unit within the genus *Onychogomphus*, sister to *O. boudoti*. This fact, together with the unique morphology of the specimens belonging to this clade, as discussed below, lead us to describe these populations as representatives of a new species.

***Onychogomphus cazuma* Barona, Cardo & Díaz sp. nov.**
(Figs 3–8)

Material studied

Holotype ♂ (MNCN_Ent 268518). España, Valencia, Bicorp, río Cazuma, 39.1108333°N, 0.853055°W (UTM 30SXJ8531), 435 m a.s.l., 20-vii-2019,

leg. J. Barona [white label, printed]; MNCN_Ent 268518 [white label, printed], Holotypus, *Onychogomphus cazuma* Barona, Cardo & Díaz des. 2020 [red label, printed]. Mature male, dry-preserved in the Entomological Collection of MNCN.

Paratypes (8♂ 3♀).

5♂ in ethanol, labelled: »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, J. Barona, N. Cardo & C. Díaz, 14-vii-2017«; MNCN_Ent 255172/ »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, Barona, Cardo, Díaz & Teruel, 08-vi-2019«; MNCN_Ent 255173/ »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, J. Barona, 05-vii-2019«; MNCN_Ent 255167/ »Rambla del Ral, Cortes de Pallás (V), 30SXJ7048 – 434 m, Cardo & Díaz, 18-vii-2019«; MNCN_Ent

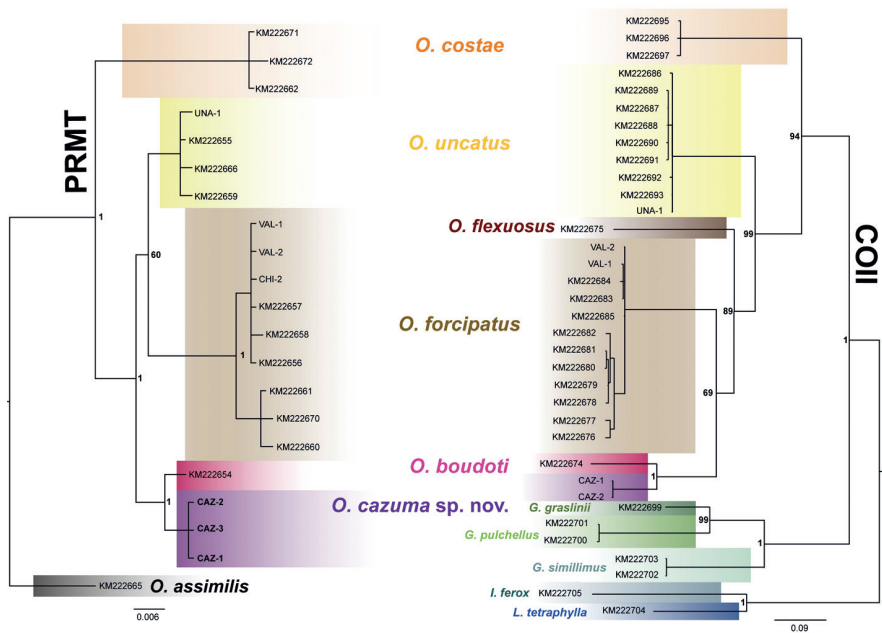


Figure 2. Molecular phylogenetic reconstruction of Western Palaearctic *Onychogomphus*. Bayesian trees obtained using MrBayes based mitochondrial (COII, right) and nuclear (PRMT, left) data. Numbers near branches represent the posterior probabilities of clades. Colour shades represent morphologically differentiated species.

255169/ »La Tosquilla, Titaguas (V), 30SXX5812, 561 m, Barona & Teruel, 07-vii-2019«; MNCN_Ent 255171 [white labels, printed; in MNCN]; 1♀ dry-preserved, labelled: »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, J. Barona, 21-vii-2019«; MNCN_Ent 268519 [white labels, printed; in MNCN]; 2♀ in ethanol, labelled: »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, J. Barona, 20-vii-2019«; MNCN_Ent 255168/ »Rambla del Ral, Cortes de Pallás (V), 30SXJ7048 - 434 m, Cardo & Díaz, 18-vii-2019«; MNCN_Ent 255170 [white labels, printed; in MNCN]; 3♂ dry-preserved, labelled: »La Tosquilla, Titaguas (V), 30SXX5812, 600 m, P. González, J.M. Michelena, 19-vi-2003«; MUVHN_ENV00018882/ »La Tosquilla, Titaguas (V), 30SXX5812, 600 m, P. González, J.M. Michelena, 24-vii-2003«; MUVHN_ENV00052471/ »La Tosquilla, Titaguas (V), 30SXX5812, 600 m, J. Baixeras, J.M. Michelena, 09-ix-2003«; MUVHN_ENV00050960 [collecting data in white labels, printed; voucher number in red labels, printed; in ICBIBE].

All paratypes labelled: »Paratypus, *Onychogomphus cazuma* Barona, Cardo & Díaz des. 2020« [red labels, printed].

Exuviae (1♂ 1♀)

Dry-preserved, labelled: »Río Cazuma, Bicorp (V), 30SXJ8531, 435 m, S. Teruel, 12-vi-2019«; MUVHN_ENV00052558/ »La Tosquilla, Titaguas (V)/30SXX5812, 600 m, J. Barona & S. Teruel, 07-vii-2019«; MUVHN_ENV00051056 [collecting data in white labels, printed; voucher number in red labels, printed; in ICBIBE].

Etymology

The epithet *cazuma* (noun in apposition and, therefore, invariable) refers to the type locality, which was also the site where we first realized that it could be a new species.

Male (holotype)

Head – Eyes light blue, when alive; frons, anteclypeus, postclypeus and labrum yellow, bordered by black; base of mandibles yellow, sclerotized extremities brown; labium yellow with median lobe bordered by brown; vertex black with a yellow rounded spot in line with the central ocellus and as

wide as it; occiput trapezium-shaped and yellow; 4-segmented antennae mainly black (flagellum and pedicel), except basal segment (scape) yellow (Fig. 3a).

Thorax – Prothorax black with yellow marks: anterior lobe black with yellow anterior margin; median lobe with four yellow spots (two central and two lateral); posterior lobe yellow divided into two pieces by a furrow, which is black at the anterior margin. Synthorax in dorsal view: the yellow collar (transverse crest at the front of synthorax dorsum) and the yellow area posterior to it, are not interrupted with black, except for a faded black dot at the junction of the dorsal carina and the collar; dorsal carina yellow, ante-alar crest black, ante-alar sinus black and yellow. Black mid-dorsal stripes on both sides of dorsal carina, yellow post-dorsal stripe is connected with yellow area adjacent to collar and with yellow ante-humeral stripe. In lateral view, mesepimeron, metepisternum and metepimeron yellow and bordered by black stripes. Between the humeral (or mesothoracic pleural) and metapleural (or metathoracic pleural) sutures, black stripes frame two characteristic yellow spots: superior hammer-shaped, and inferior bow-shaped with the metastigma in its centre. Yellow patch between the metapleural suture and the metathoracic coxa (Fig. 3b). Legs: Coxae and trochanters mainly yellow; black femora with yellow marks, the metathoracic femur has a yellow oval proximal spot at the exterior of the leg. The joint that articulates femur and tibia is yellow, and the tibiae, tarsi and pretarsi black (Fig. 3b).

Wings – Hyaline, venation dark brown except for the whitish costa of FW and HW. Pt black bordered by black veins, above 3 cells in FW and $2\frac{1}{2}$ – $3\frac{1}{2}$ cells in HW; 11 antenodal cross-veins in FW and 8 in HW; 7–8 Px in FW and 7–8 in HW; anal triangle 3-celled (Fig. 3a).

Abdomen – Black with mid-dorsal spots, yellow in S1 and S2, fading to whitish from S3 to S7, and yellow again in S8–S10, which gives the specimen a pale overall appearance. Wider at S1–S2 and from S7 to S10. Two yellow auricles in S2. In dorsal view, spot in S1 is yellow and triangular-shaped, in S2 it is elongated, and in S3–S7 are bilobed, with proximal lobes extend-

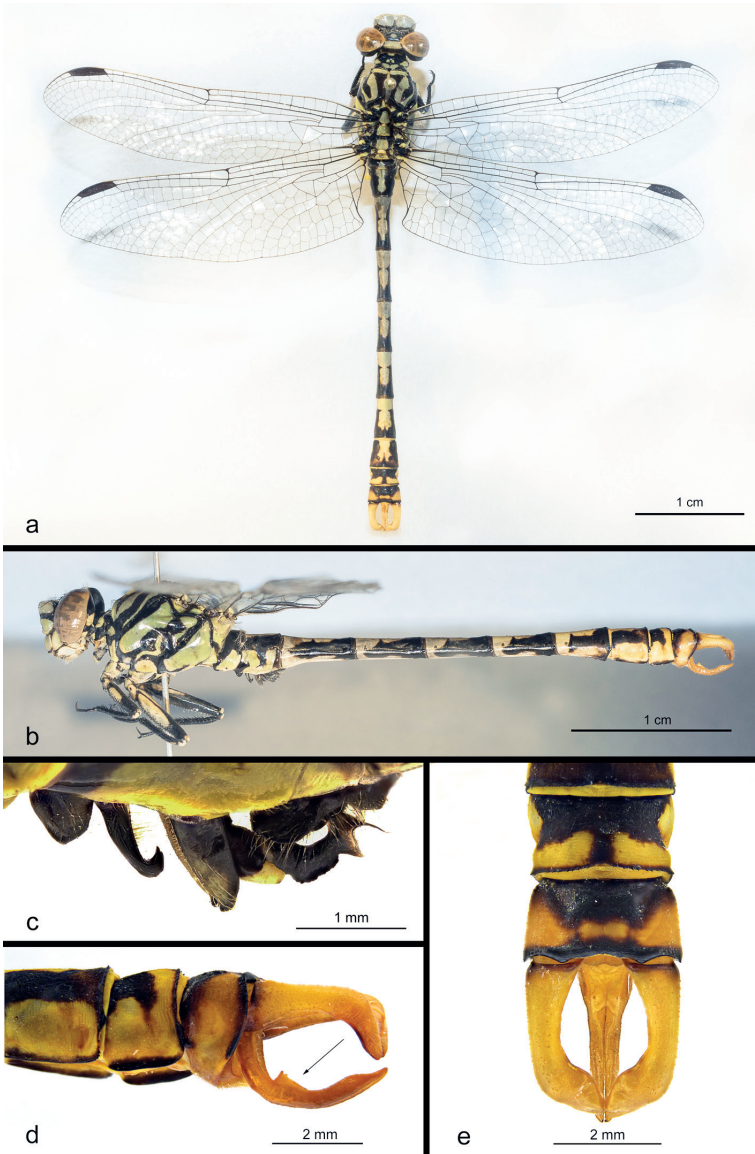


Figure 3. *Onychogomphus cazuma* sp. nov., holotype: a – dorsal view; b – lateral view; d – lateral view of anal appendages (diagnostic feature indicated by the arrow); e – dorsal view of cerci and epiproct. Paratype TOS1, (La Tosquilla): c – lateral view of accessory genitalia.

ing laterally and ventrally, and distal lobe is lanceolate, except for S7 where it is bifid. In S8 spot is bifid and does not extend laterally. In S9, a yellow band at the distal border extends laterally and ventrally, interrupted in the middle by a black line. S10 is black at the proximal end and mainly yellow at the distal end (Fig. 3a). Secondary genitalia are mainly black, with yellow spots on penis vesicle and anterior face of genital lobe. Anterior lamina rounded, posterior hamule ends in a prominent and sharp hook; oblong genital lobe is 1.5× longer than posterior hamule. Glans of penis ends in two short and straight flagella or cornua (Fig. 3c).

Anal appendages – Superior anal appendages yellow; more than twice the length of S10. Their angled distal ends do not overlap, and in distal view they present a jagged margin. Inferior anal appendage (epiproct) yellow, bifid and slightly longer than the superior anal appendages. Its distal end, curved upwards, is bezel-shaped. In sub-basal position, the epiproct has two cuticular laminar expansions that in lateral view appear as a ridge ascending towards the proximal end, culminating in a peak straight upwards (Fig. 3d, 4e). These expansions do not project outwards in dorsal view (Fig. 3e).

Measurements [mm]: Total length (frons to end of anal appendages): 45.2; Abdomen total length: 32.0; Abdomen (excluding anal appendages): 29.5; FW: 27.5; HW: 26.8; Pt length: 3.2 in FW and HW.

Variation in male paratypes

On the thorax dorsum, the black dot at the junction of the dorsal carina and the collar may be more or less marked and even absent. The shape of the markings on the sides of the thorax is slightly variable (Fig. 4d). Some males have tiny yellow markings at the proximal end of the tibiae, especially in the hind pair of legs. CAZ1 has two black symmetrical spots between the mid-dorsal and ante-humeral black stripes.

Total length varies between 43.1–46.4 mm. Ax FW 10–13, HW 7–10. Px FW 6–8, HW 8–9. Pt is above 3–4 cells in FW and HW. In HW, anal triangle has 3 cells in holotype and paratypes, but in one of the photographed individuals (Fig. 4c) has 4 cells.

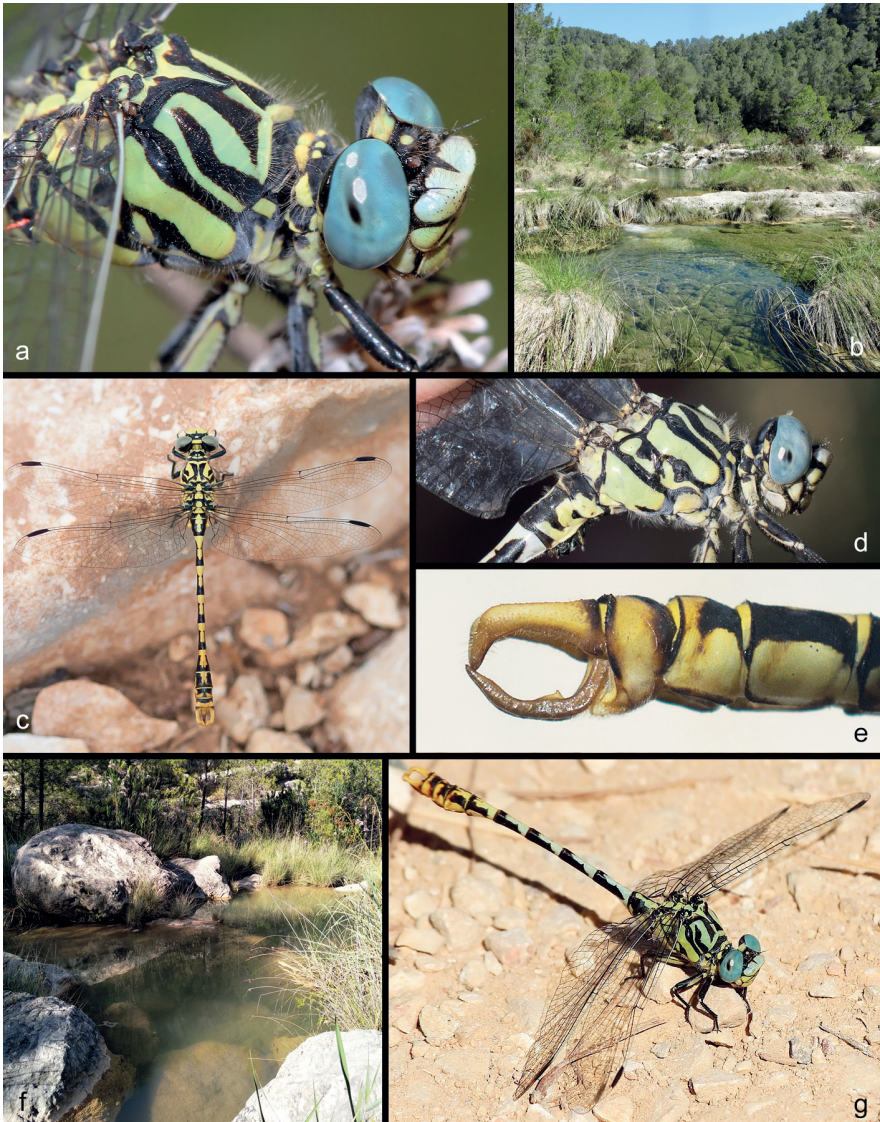


Figure 4. *Onychogomphus cazuma* sp. nov.: a – head and thorax detail (Rambla del Ral, photo: Toni Alcocer); b – type locality (Cazuma river, photo: JBF); c – habitus (Cazuma river, photo: CDM); d – lateral thorax (RAL1, Rambla del Ral, photo: CDM); e – lateral view of male genitalia (RAL1, Rambla del Ral, photo: CDM); f – Rambla del Ral (photo: CDM); g – habitus (Cazuma river, photo: Toni Alcocer).

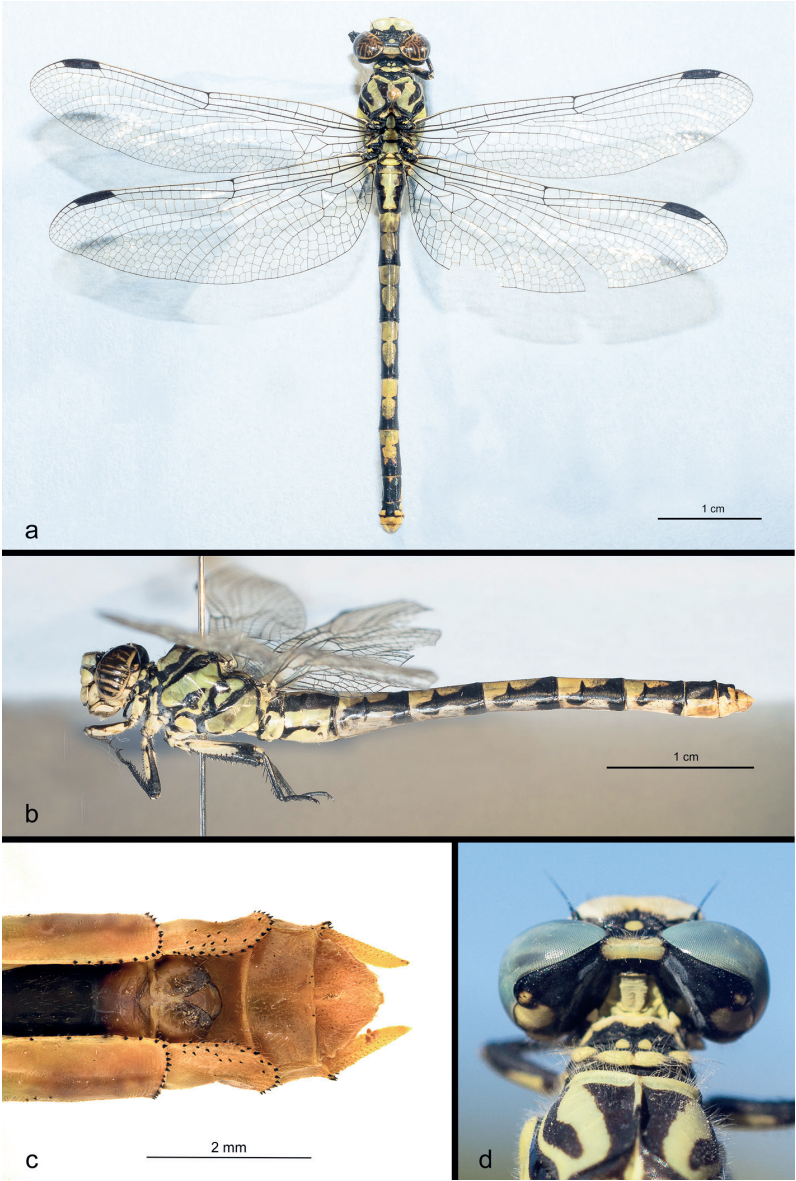


Figure 5. Female paratypes of *Onychogomphus cazuma* sp. nov., CAZ7 (Cazuma river): a – dorsal view; b – lateral view; c – ventral view with vulvar scale. RAL2 (Rambla del Ral): d – posterior view of eye (photo: CDM).

Variation in female paratypes

In lateral thorax marks, a yellow bow-shaped spot is connected to yellow mesepimeron (Fig. 5b), and a yellow hammer-shaped spot is isolated in two female paratypes (Fig. 5b) and connected to bow-shaped spot in the third one.

Female's abdominal colour pattern is similar to that of male, except for yellow spots on S7–S8: spot in S7 is not deeply divided as in males; spot in S8 is smaller than in males, variable in shape but never bifid (Fig. 5a). In females, the tibiae of the first and third pair of legs have yellow lines (Fig. 5b).

The vulvar scale (Fig. 5c) is rounded and divided into two pointed lobes by a central cleft, which is as deep as half the vulvar scale's length. Postgenae (surface posterior to the eyes) are smooth, lacking tubercles or other structures (Fig. 5d).

Total length varies between 44.0 and 47.5 mm. Ax FW 10–13; HW 8–9. Px FW 7–9; HW 6–11. Pt is above 3–4 ½ cells in FW and above 3 ½–4 cells in HW.

Exuvia

Coloration is brown with darker wing sheaths, S10 and darker paraprocts in ventral view (Figs 6a, c).

Head – wider than long, with rounded eyes and sides of occiput rounded and converging posteriorly. Four-segmented antennae; third segment is the largest, dorsoventrally flattened and concave, densely covered laterally with long setae; fourth segment tiny and conical (Fig. 6d). Prementum flat; prementum–postmentum joint reaching posterior margin of procoxae. Prementum subquadrangular, median lobe waved; its apical margin concave in the middle with a ventral row of 14–16 short and rounded teeth, as well as dorsal rows of brownish piliform setae. Labial palps curved inwards and pointed, internal margin with 10–11 teeth. Movable hook acutely pointed (Fig. 6e).

Thorax – Prothorax small, narrower than head and paler than synthorax. Divergent wing sheaths, reaching S5. Stout legs with abundant setae and spines. Femora distally with a darker marking. Pro- and mesotibiae longer

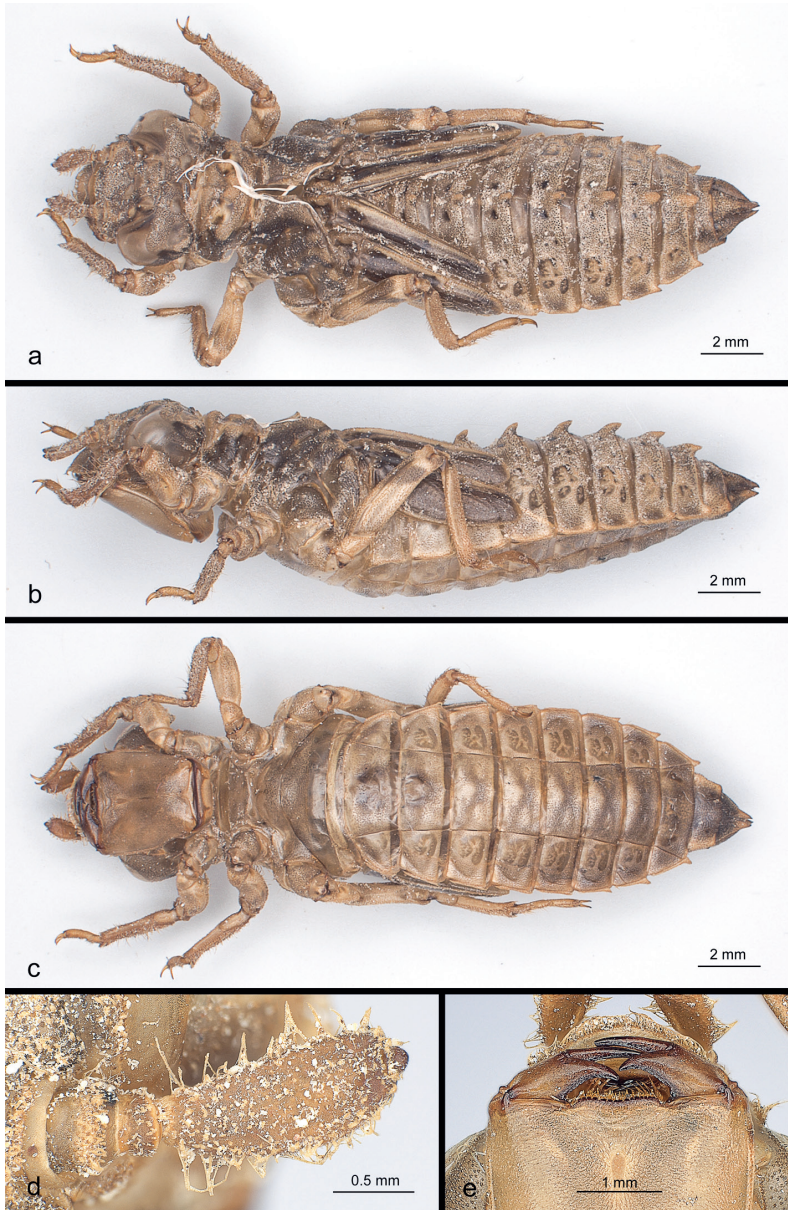


Figure 6: *Onychogomphus cazuma* sp. nov. exuviae: a – dorsal view; b – lateral view; c – ventral view; d – dorsal view of antenna; e – ventral view of prementum.

than femora, metatibiae shorter than femora. A spur is present in pro- and mesotibiae and absent in metatibiae. Light-coloured tarsi, tarsal formula 2-2-3.

Abdomen – Brown, in ventral view becoming darker distally, in dorsal view with dark markings on S2–S8, and in lateral view with dark spots on S2–S8. Mid-dorsal spines absent on S1 and S10, and prominent from S2 to S9 (Fig. 6b). Lateral spines present on S7, S8 and S9, which diverge from the edge of the segment (Fig. 6a). Anal appendages longer than S10. Epiproct triangular, slightly longer than paraprocts and longer than cerci.

Measurements [mm] – total length (including paraprocts) 20.90–22.26; third antennal segment: length 1.30–1.49 mm, width 0.54–0.60 mm; prementum: length 3.20–3.49, basal width 1.90–2.10; distal width 3.10–3.40 mm; maximum width 3.10–3.40 mm; prementum movable hook length 1.10; paraprocts length 1.10–1.20; epiproct length 1.43–1.58; cerci length 1.08–1.32 (n = 10).

Diagnosis

By its overall appearance, *Onychogomphus cazuma* sp. nov. is close to *O. uncatatus*, *O. forcipatus* and *O. boudoti*, sympatric in the Atlanto-Mediterranean region, but can be easily distinguished by morphological details. In *O. cazuma*, the male epiproct has cuticle expansions in a sub-basal position, which in *O. uncatatus* and *O. forcipatus* are tooth-shaped and project outwards in dorsal view. In *O. boudoti* and *O. cazuma* these expansions are laminar and do not project outwards, but while in *O. boudoti* they are smooth, barely prominent in lateral view, in *O. cazuma* they appear as a ridge ascending towards the proximal pole and culminating in a peak straight upwards (Fig. 7).

In females of *O. cazuma*, postgenae lack post-ocular tubercles as in *O. uncatatus*, but unlike that species the vulvar scale has two broad lobes (as in *O. forcipatus* and *O. boudoti*) divided by a median cleft. The length of this median cleft is almost as long as the vulvar scale in *O. forcipatus*, half the length of the vulvar scale in *O. cazuma*, and two-thirds of the length of the vulvar scale in *O. boudoti* (Fig. 7).

The colour pattern of *O. cazuma* differs from that of the mentioned congeners by the following combination: (i) black vertex with a rounded yellow spot, (ii) yellow thoracic collar not interrupted by a thick black line, and (iii) post-dorsal and ante-humeral yellow stripes are connected.

These features may show variability, for instance in some individuals of *O. forcipatus* and *O. cazuma* the collar may be narrowly interrupted by a

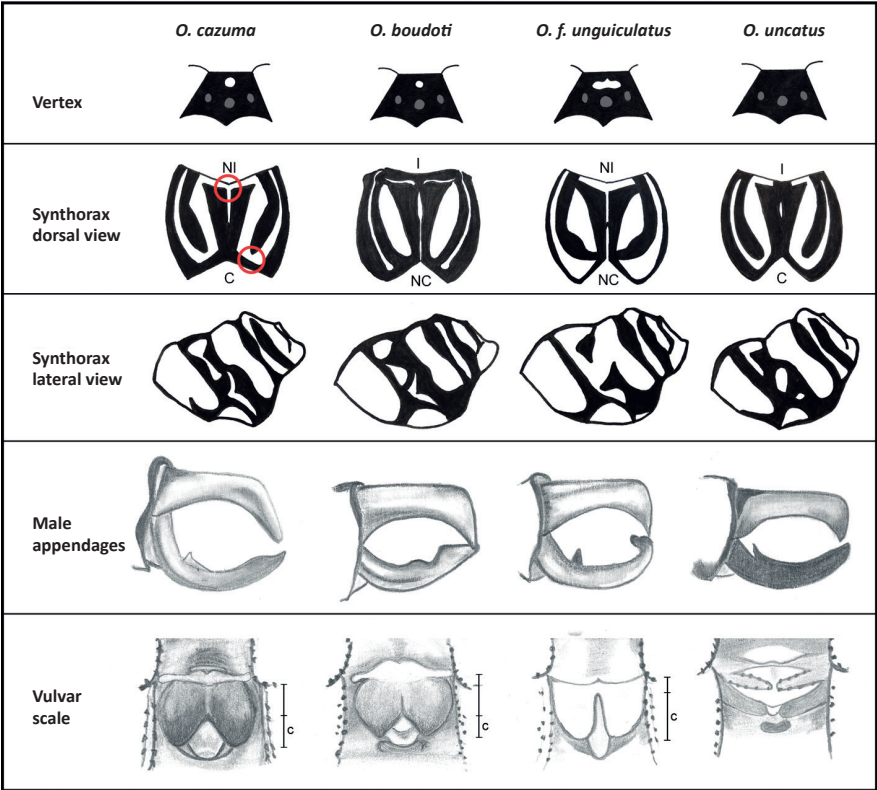


Figure 7. Ibero-Maghrebian *Onychogomphus* morphological traits: vertex (dorsal view); synthorax in dorsal view, showing interrupted (I)/not interrupted (NI) collar and connected (C)/not connected (NC) post-dorsal and ante-humeral yellow stripes; synthorax in lateral view, male appendages (lateral view) and female vulvar scale (ventral view) (the black line shows the length of the median cleft (c) in relation to the total length of the vulvar scale). Drawings: NCM, genitalia of *O. forcipatus* and *O. uncatu*s based on DIJKSTRA & LEWINGTON 2006.

slight darkening near the mid-dorsal carina. *Onychogomphus uncatus* most often has a totally black vertex, although there are rare individual exceptions, while the collar is interrupted by a thick black line. *Onychogomphus*

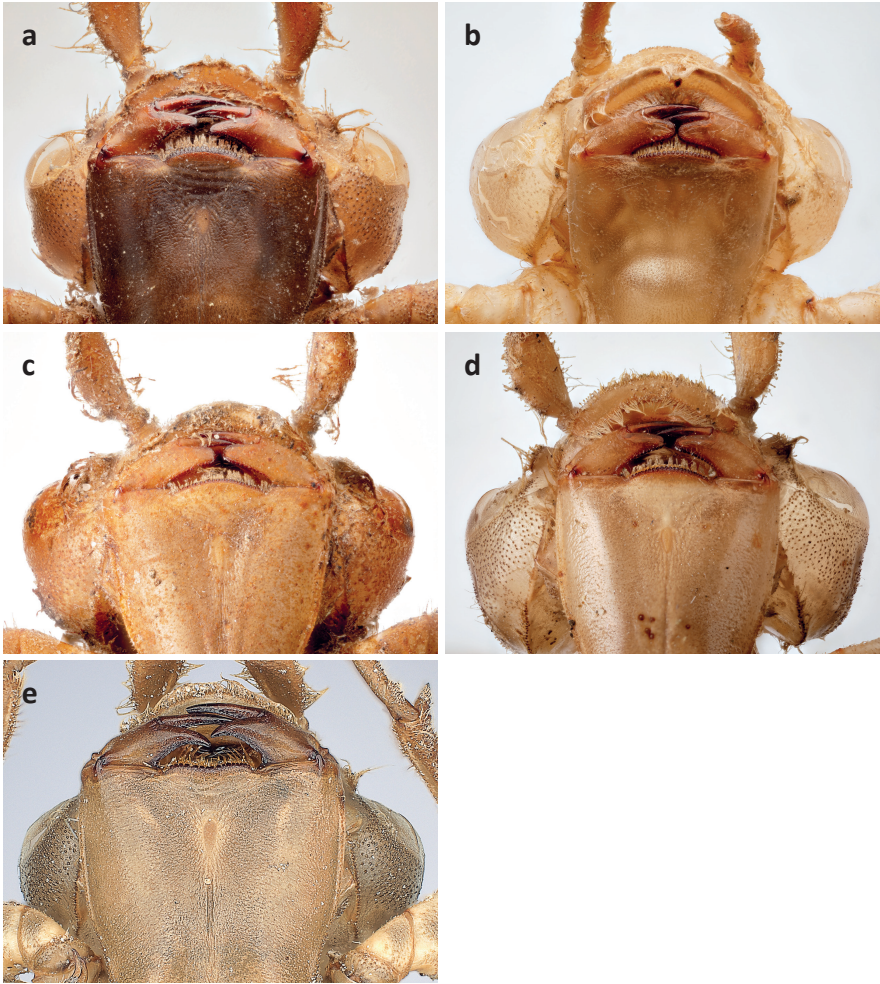


Figure 8. Prementum and labial palps of Ibero-Maghrebian *Onychogomphus* exuviae in ventral view; a – *O. boudoti*; b – *O. costae*; c – *O. forcipatus*; d – *O. uncatus*; e – *O. cazuma* sp. nov. Note the waved shape of the median lobe of the prementum and the pointed distal end of the labial palps that clearly separate *O. cazuma* from the other species. Photos: a–d by courtesy of Christophe Brochard, e – STM

forcipatus has a yellow spot on the vertex that is not round but elongate, and the post-dorsal and ante-humeral yellow stripes are not connected. *Onychogomphus boudoti* has a round yellow spot on the vertex, as in *O. cazuma*, but the collar is interrupted and the post-dorsal and ante-humeral yellow stripes are not connected (Fig. 7).

The diagnostic characters can also be used to separate the nominotypical *O. forcipatus* subspecies, *O. forcipatus forcipatus* (Linnaeus, 1758), recently recorded in the North of the Iberian Peninsula (MEZQUITA-ARAMBURU & TORRALBA-BURRIAL 2015; PAZ LEIZA et al. 2017).

Lastly, *O. cazuma* exuviae and all larval instars observed so far differ from all other regional *Onychogomphus* by the waved shape of the median lobe of the prementum and the pointed distal end of the labial palps (Fig. 8).

Ecology

Individuals of *Onychogomphus cazuma* sp. nov. were found at six watercourses in the mountainous inland part of the province of Valencia (central-eastern Iberian Peninsula, 39°N, 0.8°W, Fig. 1, Table 1), at elevations of 100–561 m a.s.l., running through Cretaceous and Jurassic limestone (GUTIÉRREZ et al. 1984). The climate is typically Mediterranean with a dry and hot summer (Csa) according to the Köppen-Geiger classification (AGENCIA ESTATAL DE METEOROLOGÍA & INSTITUTO DE METEOROLOGIA DE PORTUGAL 2011). In local weather stations, mean annual temperature ranges between 15.4–17.8°C and mean annual rainfall is 418.8–602.6 mm (SISTEMA DE INFORMACIÓN GEOGRÁFICA DE DATOS AGRARIOS 2020).

Onychogomphus cazuma inhabits springs, streams or upper courses of small lowland Mediterranean rivers (100–561 m a.s.l.) in well-preserved forest environments with barely any agriculture, livestock or habitation. These small perennial limestone rivulets are typically 1–5 m wide and 2–60 cm deep, with low flow (mean annual flow <0.1 m³/s, IGME-DGA 2009) but a torrential regime, with occasional strong floods. Water is very clean and oligotrophic; calcium bicarbonate is the main mineral present and conductivity exceeds 400 µS/cm (IGME 1988; LABORATORIO SALUD PÚBLICA 2019). The high content of calcium salts facilitates both chemical and biological precipitation of calcareous tufa including calcified plant or mollusc fragments. The alternation of low slope shallow sections, small riffles, falls

and pools up to 2–3 m deep offers macrohabitat diversity. The substrate is dominated by bedrock with variable amounts of scattered cobbles, gravels and sand, as well as limestone silt.

Aquatic vegetation is characterized by various unicellular algae growth in the riverbed and scarce macrophytes (*Chara* L., 1753, and *Nigella* L., 1753). Riparian vegetation consists of a Mediterranean riparian thicket of *Nerium oleander* L., 1753 (*Rubro ulmifolii-Nerietum oleandrii*, Bolòs, 1956), interspersed with helophytes like *Phragmites australis* (Cav.) Trin. ex Steud., 1841, *Cladium mariscus* (L.) Pohl, 1882, *Schoenus nigricans* L., 1753, and other Poaceae and Cyperaceae. Steep rocky slopes by the river are mainly occupied by Aleppo pine forests (*Pinus halepensis* Mill., 1768) and sclerophyllous scrub (*Quercus coccifera* L., 1753, *Juniperus phoenicea* L., 1753, *Pistacia lentiscus* L., 1753, *Salvia rosmarinus* (L.) Schleid., 1852, *Chamaerops humilis* L., 1753) (FERRER GALLEGO 2014).

Sections of the watercourses occupied by *O. cazuma* are very short, from 150 to 1800 m. Resident males (1–15 per section) were observed at shallow waters (<15 cm) with a low discharge, bordered by dense stands (up to 70%) of Poaceae and Cyperaceae such as *C. mariscus* or *S. nigricans*. While males could be seen perched on stones and riverine vegetation, females visit the riverbed only for mating and ovipositing. At the Cazuma river, both males and females use cleared areas (abandoned fields) far from the riverbed (30–50 m) as possible maturation areas, where they were seen perching on vegetation, stones and ground. At La Tosquilla, several males were seen perched close to each other.

A female was observed ovipositing into a pool (1 m depth) with thick (30 cm) limestone silt at La Tosquilla (07-vii-2019). Larvae were found in shallow water (<10 cm depth) and low-discharge sections, with a bottom of gravel, calcified silt and leaf litter (1–4 cm). Most exuviae were found at such sites too, clinging to herbaceous vegetation (*C. mariscus*, *S. nigricans*), 1–3 cm above the water surface. The species was in flight from 08-vi to 09-ix, with three emergences recorded between 08- and 16-vi.

The Cazuma river (Fig. 4b) has a diverse community of Odonata, including 37 species (BARONA 2017; BDB 2020). In this area *O. cazuma* is sympatric with *O. uncatatus* and *O. forcipatus*. *Onychogomphus uncatatus* is also present in La Tosquilla and the Fraile river. Notable is the overlap of *O. ca-*

zuma and *Oxygastra curtisii* (Dale, 1834) at all studied sites except Rambla del Ral (Fig. 4f) (BDB 2020).

The possible ecological segregation of *O. cazuma* and *O. uncatius* was observed in the Cazuma river, where *O. cazuma* was seen at sections with slower flow and denser riparian vegetation, and in La Tosquilla, where *O. cazuma* was present at a spring while *O. uncatius* inhabited the Turia river.

A female photographed in 2008 at La Tosquilla was trapped in an orb-weaver spider (*Larinioides* Caporiacco, 1934) web. A teneral specimen was collected on 08-vi-2019 in Río Cazuma while it was devoured by a male of *Gomphus graslinii* Rambur, 1842.

Discussion

The discovery of a new species of dragonfly in Europe is a surprise. The European fauna of Odonata is well-known, with just a few additions of new species in the last five decades, viz. *Somatochlora borisi* Marinov, 2001, *Cordulegaster helladica* (Lohmann, 1993), *C. heros* Theischinger, 1979, and *C. trinacriae* Waterston, 1976. Furthermore, with *Ischnura graellsii* Rambur, 1842, the last new species described from Spain dates from the middle of the 19th century.

The morphology of the male appendages and female vulvar scale suggests a close relationship between *Onychogomphus cazuma* sp. nov. and the Moroccan *O. boudoti*. Molecular phylogenetic reconstruction concurs with this morphological evidence, indicating a sister relationship between *O. cazuma* and *O. boudoti*. The discovery of *O. cazuma* clarifies the phylogenetic position of the Maghrebian *O. boudoti*, demonstrating the shared evolutionary history of the south-western European and the North African odonate species in the Western Palaearctic. Both *O. boudoti* and *O. cazuma* are restricted to small geographic areas (cf. FERREIRA et al. 2014b; VAN DER MEER 2018).

Onychogomphus cazuma lives under similar conditions, including places where emergence was observed, to those described for *O. boudoti* (FERREIRA et al. 2014b). We observed that in sympatry with other congeners, *O. cazuma* seemed to prefer river sections with slower flow and more vegetated banks, with helophyte and grass cover; but more extensive ecological research is needed to confirm these preferences.

So far, *O. cazuma* has been only located within 'lowland Mediterranean rivers' (see TORO et al. 2009 for the typology). This riverine habitat is found along the Mediterranean Spanish coast from Girona to Cádiz, in the Ebro and Guadalquivir basins and on the island of Mallorca. It is possible that populations of *O. cazuma* could be present at more sites within this wider area.

The close relationship between *O. cazuma* and *O. boudoti* allows us to speculate that the geographic distribution of their presumed common ancestor could have dwelled in similar habitats of both the Iberian Peninsula and the North of Morocco.

Conservation of the Iberian endemic *Onychogomphus cazuma*

Onychogomphus cazuma sp. nov. was found in relatively well-preserved areas, although these are not exempt from human disturbance. The main threats are water abstraction for domestic use and/or irrigation and recreational activities (like swimming or canyoning). Given the small size and low flows of these watercourses, any increase of use can make a substantial difference in the availability of habitat for this species.

Furthermore, *O. cazuma*, as the only dragonfly endemic to Iberia (cf. KALKMAN et al. 2010), may need special protection measures. Following the IUCN Red List Criteria (IUCN STANDARDS AND PETITIONS SUBCOMMITTEE 2019), we have determined that the known extent of occurrence (EOO) of the species is 1273 km² and the known area of occupancy (AOO) is 32 km². Although the species was recorded at six sites, we consider only five locations (*sensu* IUCN). In these sites, the water level and seasonality are heavily dependent on the groundwater table, some of them sharing the same local aquifer – for instance the Cazuma river-Manyana and Fraile-Grande rivers (IGME 1988; IGME-DGA 2009). Moreover, in recent decades the length of the Cazuma river that is remaining dry all year (except after heavy rain events) has substantially increased, suggesting a decline in extent and quality of this habitat. However, we assume that the species could be more widely distributed than currently known. Therefore we suggest for the moment that *O. cazuma* should be considered as »Data Deficient« by the criteria of the IUCN Red List of Threatened Species. Further research is essential to establish the species' status and identify its conservation needs.

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References

- AGENCIA ESTATAL DE METEOROLOGÍA & INSTITUTO DE METEOROLOGIA DE PORTUGAL 2011. Atlas Climático Ibérico. Temperatura del aire y precipitación (1971–2000). Ministerio de Medio Ambiente y Medio Rural y Marino e Instituto de Meteorologia de Portugal, Madrid-Lisboa
- BARONA J. 2017. Distribución, abundancia y estado de conservación de odonatos amenazados en la cuenca del río Escalona y su entorno (Canal de Navarrés, macizo del Caroche). CNME 17/0301/16. Unpublished report, Servicio de Vida Silvestre, DG de Medi Natural i d'Avaluació Ambiental, Generalitat Valenciana
- BDB 2020. Banco de Datos de Biodiversidad de la Comunitat Valenciana. Online on the internet, URL [03-iii-2020]: <http://www.bdb.gva.es/>
- BOUDOT J.-P. & KALKMAN V.J. (Eds) 2015. Atlas of the European dragonflies and damselflies. KNNV Publishing, Zeist
- BYBEE S.M., OGDEN T.H., BRANHAM M.A. & WHITING M.F. 2008. Molecules, morphology and fossils: a comprehensive approach to odonate phylogeny and the evolution of the odonate wing. *Cladistics* 24: 477-514
- CANO VILLEGAS F.J. 2009. Desarrollo larvario de *Onychogomphus costae* Sélys, 1885 en el sur de la Península Ibérica y aclaración sobre su confusión con *Ophiogomphus cecilia* (Fourcroy, 1785) (Odonata: Gomphidae). *Boletín de la Sociedad Entomológica Aragonesa* 44: 327-332
- CARLE F.L., KJER K.M. & MAY M.L. 2015. A molecular phylogeny and classification of Anisoptera (Odonata). *Arthropod Systematics & Phylogeny* 73: 281-301
- DIJKSTRA K.-D.B. & KALKMAN V.J. 2012. Phylogeny, classification and taxonomy of European dragonflies and damselflies (Odonata): a review. *Organisms Diversity & Evolution* 12: 209-227
- DIJKSTRA K.-D.B. & LEWINGTON R. 2006. Field guide to the dragonflies of Britain and Europe. British Wildlife Publishing, Gillingham
- FERREIRA S., LORENZO-CARBALLA M.O., TORRES-CAMBAS Y., CORDERO-RIVERA A., THOMPSON D.J. & WATTS P.C. 2014a. New EPIC nuclear DNA sequence markers to improve the resolution of phylogeographic studies of coenagrionids and other odonates. *International Journal of Odonatology* 17: 135-147
- FERREIRA S., VELO-ANTON G., BROCHARD C., VIEIRA C., CELIO ALVES P., THOMPSON D.J., WATTS P.C. & BRITO J.C. 2014b. A critically endangered new dragonfly species from Morocco: *Onychogomphus boudoti* sp. nov. (Odonata: Gomphidae). *Zootaxa* 3856 (3): 349-365
- FERRER GALLEGO P.P. 2014. Estudio crítico de la flora vascular del Lugar de Interés Comunitario "Muela de Cortes y Caroche" (Valencia). Bases para su gestión y conservación. PhD Thesis, Universidad de Valencia
- FERRERAS-ROMERO M., ATIÉNZAR M.D. & CORBET P.S. 1999. The life cycle of *Onychogomphus uncatus* (Charpentier, 1840) (Odonata: Gomphidae) in the Sierra Morena Mountains (southern Spain): an example of protracted larval development in the Mediterranean basin. *Archiv für Hydrobiologie* 144: 215-228
- GUTIÉRREZ G., ELÍZAGA E., GOY J.L., NIETO M. & ROBLES F. 1984. Mapa geológico de la provincia de Valencia. Escala 1:200.000.

Ed. Diputación Provincial de Valencia, Universidad de Valencia e Instituto Geológico y Minero de España

IGME. 1988. Las aguas subterráneas en la Comunidad Valenciana. Uso, calidad y perspectivas de utilización. Instituto Geológico y Minero de España. Madrid

IGME-DGA. 2009. Identificación y caracterización de la interrelación que se presenta entre aguas subterráneas, cursos fluviales, descarga por manantiales, zonas húmedas y otros ecosistemas naturales de especial interés hídrico. Demarcación Hidrográfica 081 Júcar. Memoria Resumen. Instituto Geológico y Minero. Dirección General del Agua. Ministerio de Ciencia e Innovación-Ministerio de Medio Ambiente y Medio Rural y Marino, Gobierno de España

IUCN STANDARDS AND PETITIONS COMMITTEE. 2019. Guidelines for using the IUCN Red List categories and criteria. Version 14. Prepared by the Standards and Petitions Committee. Online on the internet, URL [04-ii-2020]: <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>

KALKMAN V.J., BOUDOT J.-P., BERNARD R., CONZE K.-J., DE KNIJF G., DYATLOVA E., FERREIRA S., JOVIĆ M., OTT J., RISERVATO E. & SAHLÉN G. 2010. European Red List of dragonflies. Publications Office of the European Union, Luxembourg

KALKMAN V.J., CLAUSTNITZER V., DIJKSTRA K.-D.B., ORR A.G., PAULSON D.R. & VAN TOL J. 2008. Global diversity of dragonflies (Odonata) in freshwater. *Hydrobiologia* 595: 351-363

KATO H., ROZEWICKI J. & YAMADA K.D. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160-1166

LABORATORIO DE SALUD PÚBLICA DE VALENCIA 2019. Informe de análisis físico-químico a la salida del manantial del Nacimiento del Río Cazuma (Bicorp, Valencia). Unpublished report, Conselleria de Sanitat Universal i Salut Pública. Generalitat Valenciana

MEZQUITA-ARAMBURU I. & TORRALBA-BURRIAL A. 2015. Primera cita de *Onychogomphus forcipatus forcipatus* (Linnaeus, 1758) (Odonata: Gomphidae) para la Península Ibérica. *Boletín de la Sociedad entomológica aragonesa* 57: 365-366

MILLER M.A., PFEIFFER W. & SCHWARTZ T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans (Louisiana): 1-8

PAZ LEIZA L., CONESA GARCÍA M. & TORRALBA BURRIAL A. 2017. Contribución de la red de seguimiento de la calidad de los ríos gipuzcoanos al conocimiento de la distribución de los Odonata de Gipuzkoa (España). *Boletín de la Sociedad entomológica aragonesa* 61: 278-280

PRUNIER F. 2018. *Onychogomphus costae* in Andalusia, southern Spain – mapping an overlooked species (Odonata: Gomphidae). *Odonatologica* 47: 1-22

REHFELDT G. 2004. Diel pattern of activity, mating, and flight behaviour in *Onychogomphus uncatatus* (Odonata: Gomphidae). *International Journal of Odonatology* 7: 65-71

RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD L.A. & HUELSENBECK J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542

SCHORR M. & PAULSON D. 2020. World Odonata List. Online on the internet, URL [22-iv-2020]: <https://www.pugetsound.edu/files/resources/world-odonata-list-67.xls>

SISTEMA DE INFORMACIÓN GEOGRÁFICA DE DATOS AGRARIOS. Ministerio de Agricultura, Pesca y Alimentación, Ministerio para la Transición Ecológica. Gobierno de España. Online on the Internet, URL [03-iii-2020]: <https://sig.mapama.gob.es/siga/>

TORO M., ROBLES S., TEJERO I., CRISTÓBAL E., VELASCO S., SÁNCHEZ J.R. & PUJANTE A. 2009. 3200. Tipo Ecológico Nº 9. Ríos mineralizados de baja montaña mediterránea. In: VV.AA. (Ed.), Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid

VAN DER MEER H. 2018. Notities over het gedrag van *Onychogomphus boudoti* in Marokko en een tweede vindplaats. *Brachytron* 19: 93-99

VELASCO-VILLANUEVA T., CAMPOS F., NORLING U. & FERRERAS-ROMERO M. 2018. The life cycles of *Boyeria irene* and *Onychogomphus uncatus* (Odonata: Aeshnidae, Gomphidae) in western Spain: A biometric study. *European Journal of Entomology* 115: 684-696

Appendix 1. Supplementary Table: GenBank sequences used in this study.

Voucher number	ID	Reference	GenBank accession code	
			COII	PRMT
Df1089_PRT	<i>Gomphus graslinii</i>	FERREIRA et al. (2014b)	KM222699	–
Df1090_PRT	<i>Gomphus pulchellus</i>	FERREIRA et al. (2014b)	KM222700	–
Df451_PRT	<i>Gomphus pulchellus</i>	FERREIRA et al. (2014b)	KM222701	–
Df607_PRT	<i>Gomphus simillimus</i>	FERREIRA et al. (2014b)	KM222702	–
Df606_PRT	<i>Gomphus simillimus</i>	FERREIRA et al. (2014b)	KM222703	–
Df1732_UGA	<i>Ictinogomphus ferox</i>	FERREIRA et al. (2014b)	KM222705	–
Df1749_MKD	<i>Lindenia tetraphylla</i>	FERREIRA et al. (2014b)	KM222704	–
Df269	<i>Onychogomphus assimilis</i>	FERREIRA et al. (2014b)	KM222694	KM222665
Df1028_MAR	<i>Onychogomphus boudoti</i>	FERREIRA et al. (2014b)	KM222674	KM222654
Df1763_MAR	<i>Onychogomphus costae</i>	FERREIRA et al. (2014b)	KM222695	KM222672 KM222671
Df489_MAR	<i>Onychogomphus costae</i>	FERREIRA et al. (2014b)	KM222696	–
Df488_MAR	<i>Onychogomphus costae</i>	FERREIRA et al. (2014b)	KM222697	KM222662
Df1730_TUR	<i>Onychogomphus flexuosus</i>	FERREIRA et al. (2014b)	KM222675	–
Df1133_MAR	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222676	KM222656
Df117_ITA	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222677	KM222656
Df775_GRC	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222678	KM222661
Df241_ITA	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222679	KM222660
Df268_ARM	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222680	KM222661
Df776_GRC	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222681	KM222660
Df467_RUS	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222682	KM222670
Df242_ITA	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222683	KM222660
Df624_PRT	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222684	KM222656
Df1767_FRA	<i>Onychogomphus forcipatus</i>	FERREIRA et al. (2014b)	KM222685	KM222656
Df281_ITA	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222686	KM222666
Df1751_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222687	KM222659
Df1752_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222688	KM222659
Df1753_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222689	KM222659
Df1754_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222690	KM222659
Df1756_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222691	KM222659
Df1750_MAR	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222692	KM222659
Df1083_PRT	<i>Onychogomphus uncatus</i>	FERREIRA et al. (2014b)	KM222693	KM222655