Zootaxa 3911 (3): 381–395
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3911.3.5
http://zoobank.org/urn:lsid:zoobank.org:pub:4E11C1AB-2614-4C4A-809D-EF7C5BE959D9

New species of Limnephilidae (Insecta: Trichoptera) from Europe: Alps and Pyrenees as harbours of unknown biodiversity

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Abstract

New species are described from the genera *Consorophylax* and *Anisogamus* (Trichoptera, Limnephilidae, Limnephilinae, Stenophylacini). Additionally the larva of the genus *Anisogamus*, and the larval stages of *Anisogamus waringeri* **sp. nov.** and *A. difformis* (McLachlan 1867) are described. The new species *Consorophylax vinconi* **sp. nov.** is a microendemic from the Southern Alps and differs from its congeners in the shape of the parameres, which are distinctly straitened in the distal quarter in the new species. The new species *Anisogamus waringeri* **sp. nov.** represents the second species in the hitherto monospecific genus *Anisogamus*. Compared to *Anisogamus difformis*, the male of *A. waringeri* **sp. nov.** has more-slender superior appendages; a more-rounded basal plate of the intermediate appendages, lacking pointed protuberances; and parameres shorter than the aedaegus, proximally with one dorsal and several ventral tines. Further, the two species are disjunctly distributed in the European mountain ranges (*A. difformis*: Alps, *A. waringeri* **sp. nov.**: Pyrenees). Larvae of species in the genus *Anisogamus* are characterized by the lack of a dorsal protuberance on abdominal segment I, a unique feature among Eurasian Limnephilidae. *Anisogamus difformis* and *A. waringeri* **sp. nov.** larvae differ in pronotum shape. The discovery of two new species demonstrates the significance of taxonomic studies in Europe, and the importance of adequate training for young scientists in order to assess an incompletely described biodiversity under threat of extinction.

Key words: endemism, species description, *Consorophylax*, *Anisogamus*, caddisflies

Introduction

Both the Alps and the Pyrenees are centres of biodiversity in Europe. Particularly patterns of plant, vertebrate and terrestrial invertebrate diversity in European alpine ecosystems have been extensively studied (e.g., Wohlgemuth 2002; Nagy *et al.* 2003; Iserbyt *et al.* 2008; Huemer 2011). Increasingly, aquatic invertebrates (and EPT-taxa in particular) have become the focus of attention in both the Alps and the Pyrenees (e.g., Sipahiler 1999, 2000; Graf 2005; Graf *et al.* 2008a; Malicky 2004, 2008; Brown *et al.* 2009). The genus *Consorophylax* Schmid 1955 currently comprises seven cold-stenotherm species (Malicky 2004, 2008). Larvae of the genus prefer crenal to epirhithral segments of alpine to montane springs and brooks, and mainly behave as shredders (Graf *et al.* 2008b). *Consorophylax* species show a complex distribution pattern, with several microendemics and two widespread species inhabiting the majority of the Alpine arc. In particular, the southern slopes of the Alps can be identified as centres of species richness in the genus, as microendemics have been found exclusively on the southern slopes of both the Western and Eastern Alps (Kimmins & Botosaneanu 1967; Graf *et al.* 2008b).

The genus *Anisogamus* McLachlan 1874 is currently represented by a single species, *A. difformis* (McLachlan 1867). The species is known predominantly from the Alps, but has also been recorded in the Pyrenees. As the larva was hitherto not described, ecological parameters of adult collection points indicated a cold-stenotherm species

Accepted by J. Morse: 11 Dec. 2014; published: 20 Jan. 2015

with a preference for the crenal and epirhithral segments of alpine to montane springs and brooks (Graf *et al.* 2008b).

During extensive collections in 2012 and 2013, WG recovered several male adult and larval specimens of putative *A. difformis* from the Pyrenees. Also, undescribed *Consorophylax* specimens were collected in 2012 by Gilles Vinçon, Grenoble, France. Using a classical taxonomic approach we were able to identify the specimens as new species, *A. waringeri* **sp. nov.** and *C. vinconi* **sp. nov.** Additionally, genetic sequence data were used to delineate species of *Anisogamus* and to affiliate the larva of *A. waringeri* **sp. nov.** with its identifiable adult.

In this paper, we describe the two new species of these genera *Consorophylax* and *Anisogamus* (Limnephilidae, Limnephilinae, Stenophylacini), the larval stage of the genus *Anisogamus*, and the larvae of *A. difformis* and *A. waringeri* **sp. nov.** We also provide a re-description of *A. difformis*.

Material and methods

Adult specimens were collected using sweep nets, larvae were collected by handpicking. Collected specimens were stored in 70% and 96% EthOH, for morphological and molecular analyses, respectively. Larvae of *A. difformis* were fixed and stored in formaldehyde for a prolonged period, preventing molecular analysis of those specimens.
Morphological characteristics of male genitalia were examined in KOH-treated, cleared specimens. Comparative material from the authors' collections enabled the identification of new species. Nomenclature of male genitalia follows Nielsen (1957, for *Stenophylax stellatus* (Curtis 1834), synonym of *Potamophylax latipennis* Curtis 1834). Larval morphological features are named following Wiggins (1998) and Waringer & Graf (2011), nomenclature of primary setae and setal areas follows Wiggins (1998). Illustrations were prepared according to Thomson & Holzenthal (2010): Briefly, pencil drawings of the cleared specimens were produced using a camera lucida mounted on a compound microscope, and digitally edited and "inked" with Adobe Illustrator (v. 16.0.4, Adobe Systems Inc.)

In addition to morphological features, molecular genetic sequence data were compared in order to delineate two *Anisogamus* species and to support larval association in *Anisogamus* **sp. nov.** DNA extraction and amplification of the 658-bp-long "barcode region" of the mitochondrial cytochrome oxidase *c* subunit I (mtCOI) using primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994) and the 541-bp-long fragment of the same gene using primers S20 and Jerry (Simon *et al.* 1994; Pauls *et al.* 2006) were performed as outlined by Pauls *et al.* (2008); Previšić *et al.* (2009) and Gibson *et al.* (2010). Sequences were edited manually using the program Geneious R7 (Biomatters 2014) and aligned using MAFFT (Katoh & Standley 2013). Sequences were deposited in GenBank under Accession nos: KP174658-KP174663. Inter- and intraspecific genetic distances (uncorrected *p*-distances) were calculated in Mega 4.0.1 (Tamura *et al.* 2007).

Consorophylax vinconi sp. nov. Graf & Malicky

Figs. 1A–D, 2

Holotype. 1 male: Italy, Torino, Valchiusella, Fondo, Burdeiver brook (45°30'59.60"N 7°39'09.72"E), 1800–1900 m a.s.l., 01.ix.2012, leg. G. Vinçon. Holotype deposited in the Biologiezentrum des Oberösterreichischen Landesmuseums, Linz, Austria. Paratype. 1 male, same collection date, in coll. Malicky.

Diagnosis. The new species is a *Consorophylax* most similar to *C. piemontanus* Botosaneanu 1967 (in Kimmins & Botosaneanu 1967), but exhibiting (1) parameres that are distinctly constricted in the distal quarter, with terminal spines, and (2) inferior appendages that are slightly bifurcated in lateral view and with a sharp median tip in caudal view. *Consorophylax piemontanus* has parameres tapered, lacking terminal spines; inferior appendages are not bifurcate in lateral view and with a rounded median tip in caudal view.

Description. General appearance light brown (in alcohol), tergites and sternites light brown; cephalic and thoracic setal areas cream-coloured; cephalic, thoracic and abdominal setation light brown; legs light brown; haustellum and intersegmental integument cream-coloured; wings light brown, translucent, setation on veins and membrane light brown, length of each forewing 15 mm. Male maxillary palps each trisegmented, tibial spur fomula 2,3,4.

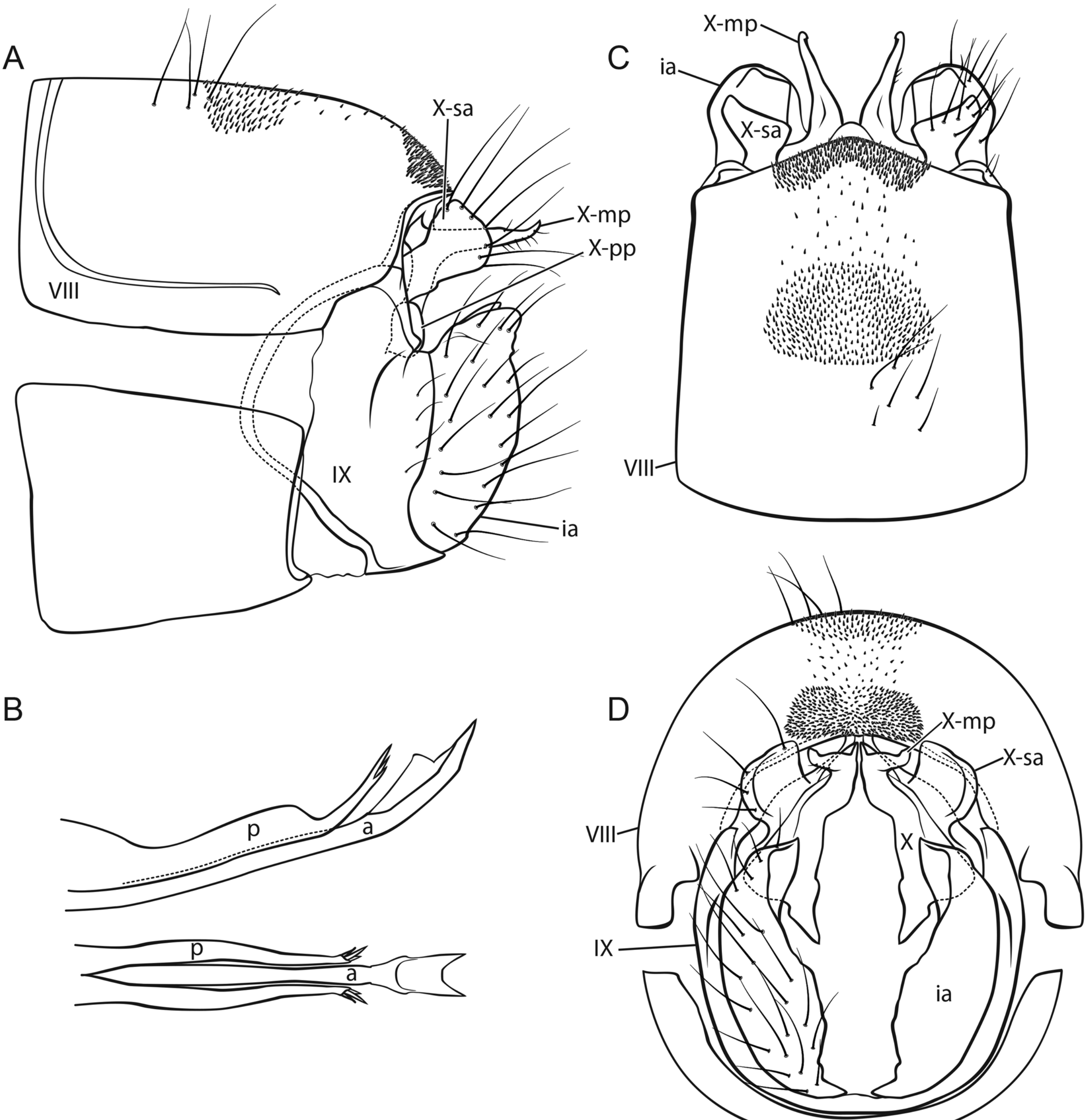

FIGURE 1. Male genitalia of *Consorophylax vinconi* **sp. nov.** A, left lateral; B, phallic apparatus, left lateral (upper) and dorsal; C, dorsal; D, caudal. Abbreviations: VIII = abdomen VIII; IX = abdomen IX; X = segment X; X-sa = lateral process of segment X ('superior appendage'); X-mp = median process of segment X ('intermediate appendage'); X-pp = posterior process of segment X; ia = inferior appendage; p = paramere; a = aedeagus.

Male genitalia (Figs. 1A–D). Tergite VIII (VIII) light brown, with median circular area of spines extending to bilobed caudal area of spines. Dorsal third of segment IX (IX) reduced to narrow transverse bridge, ventral 2/3rds broad, with distinct triangular anterior protuberance in lateral view. Lateral processes of segment X ("superior appendages", X-sa) in lateral view capitate, with small proximal ventral bulge; in dorsal view subtriangular; in caudal view subrectangular, medially concave. Sclerite of segment X (X) divided into clearly separate vertical plates on either side of phallocrypt and between lateral processes, each bearing 1 long subhorizontal, median process ("intermediate appendage", X-mp) from dorsal end, in lateral and dorsal views this process forming distinctly tapering caudad rod with sharp tip curved dorsad; posterior process on each sclerite of segment X (X-pp) in lateral view forming rounded bulge visible between lateral processes of segment X and inferior appendages (ia), in caudal view ventral part of each half of segment X subtriangular with rounded posterior process directed somewhat laterad and median process pointed toward viewer. Inferior appendages in lateral view broad, stout, each with dorsal portion directed somewhat caudad and consisting of median and lateral tip; in dorsal view stout; in caudal view dorsal portion with sharp median tip and blunt lateral tip. Aedeagus (a) slender, in lateral view curved dorsad, in dorsal view tip bifurcate. Parameres (p) in lateral view basally broad, each abruptly constricted in distal quarter to form slender, dorsally curved tip bearing 3-4 small spines.

Female, pupa, larva, and egg unknown.

Etymology. Named for the French entomologist Gilles Vinçon.

Distribution & biogeography of *Consorophylax* species. The genus *Consorophylax* has a strictly European alpine distribution. Most species are restricted to small areas, like *C. carinthiacus* Malicky 1992 (Karnische Alpen, southern Alps), *C. corvo* Malicky 2008 (Piemont, western Alps), *C. delmastroi* Malicky 2004 (Piemont, western Alps), *C. montivagus* (McLachlan 1867) (Koralpe, Saualpe, southeastern Alps), *C. piemontanus* (Piemont, western Alps) (Fig. 2). *Consorophylax styriacus* Botosaneanu (in Kimmins & Botosaneanu 1967) (eastern Alps) and *C. consors* (McLachlan 1880) (western Alps) have a slightly broader distribution within the Alpine arc (Fig. 2). Although we do not know the exact distribution range, *C. vinconi* **sp. nov.** is possibly another microendemic species within the genus. *Consorophylax vinconi* **sp. nov.** is most similar to *C. piemontanus*, which was described from Avigliana, some 13 km west of Torino. Interestingly, the type locality of *C. vinconi* **sp. nov.** is about 56 km north-northeast from the type locality of *C. piemontanus*. This supports the interpretation of *C. vinconi* **sp. nov.** as an alpine microendemic.

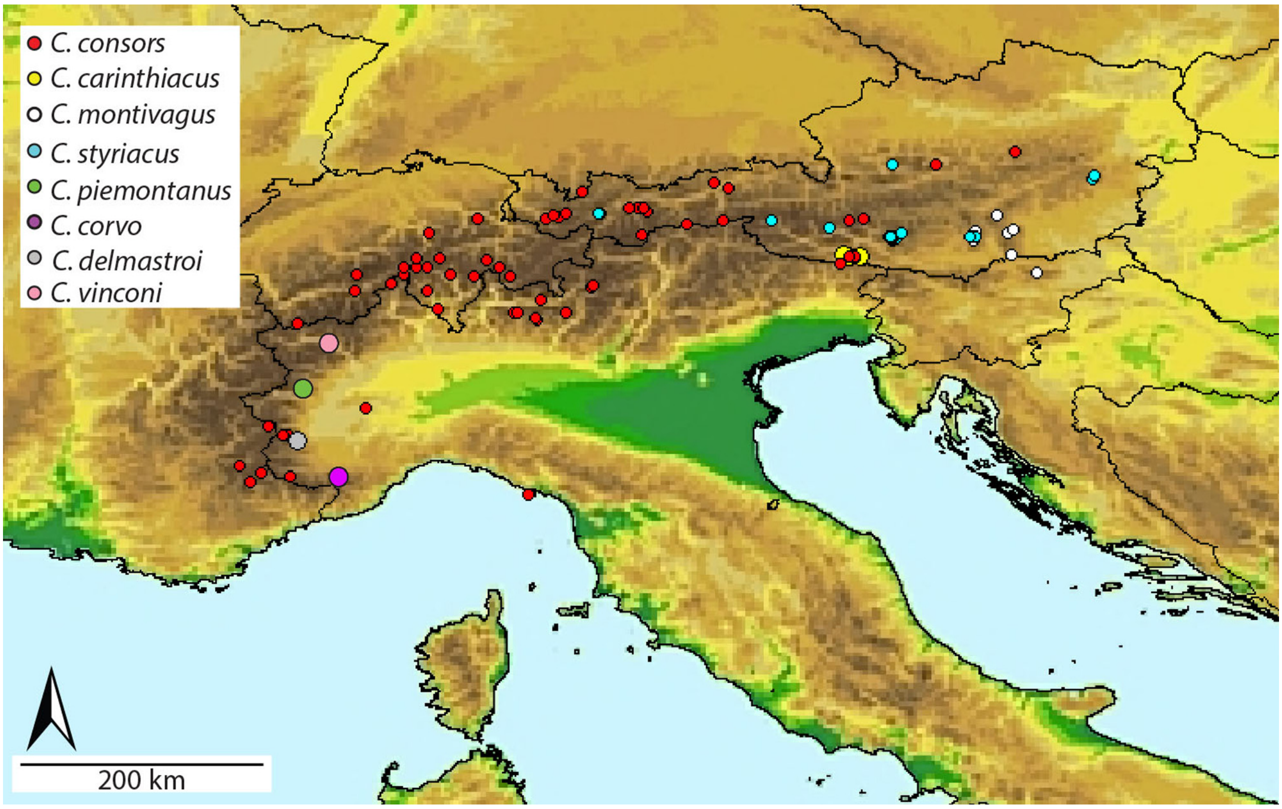

FIGURE 2. Known distributions of *Consorophylax* species.

***Anisogamus waringeri* sp. nov. Graf & Vitecek**
Figs. 3A–D, 4A–D

Holotype. 1 male, France, Pyrenées-Orientales, Mont Canigou, Col de Jou, Refuge de Mariailles (42°29'6.21"N 002°24'48.31"E) 12.vii.2012, leg. W. Graf. Holotype deposited in the Biologiezentrum des Oberösterreichischen Landesmuseums, Linz, Austria. Paratypes: 8 males, same location, 12.vii.2012, 13.vii.2013, leg. Graf.

FIGURE 3. Habitus images and known distributions of *Anisogamus* species. A, male *A. waringeri* **sp. nov.**, right lateral; B, male *A. difformis*, right lateral; C, shorter-winged female of *A. waringeri*, right lateral; D, map of the known geographical distributions of *Anisogamus* species. Records of *Anisogamus* sp. from the Pyrenees are those of Décamps (1967). *Anisogamus waringeri* **sp. nov.** was recorded from the Pyrenees by Menéndez & González (2009) as *A. difformis* (pers. comm. M. A. González).

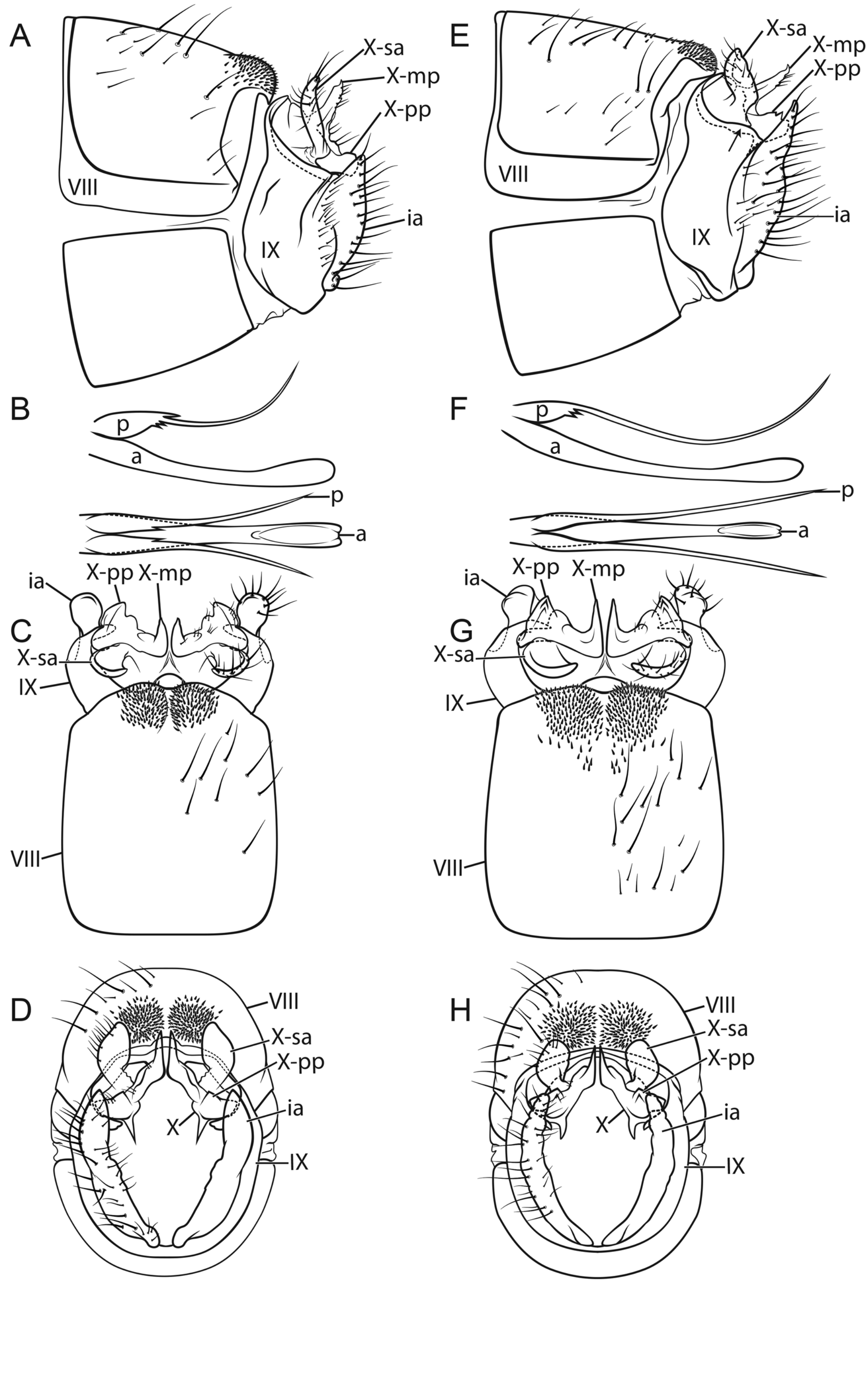

FIGURE 4. Male genitalia of *Anisogamus* species. A–D, *A. waringeri* **sp. nov.**: A, left lateral; B, phallic apparatus, left lateral (upper) and, dorsal; C, dorsal; and D, caudal. E–H, *A. difformis*: E, left lateral; F, phallic apparatus, left lateral (upper) and dorsal; G, dorsal; and H, caudal. Abbreviations as in Fig. 1.

Diagnosis. *Ansiogamus waringeri* **sp. nov.** has the following combination of male genitalia characters: (1) lateral processes of segment X ("superior appendages") slender, tall; (2) median processes of segment X ("intermediate appendages") with tips projecting dorsad, posterior processes of segment X in lateral view forming a rounded hump, discernible in caudal and dorsal view as a sharp ridge; (3) general appearance of inferior appendages stout, in lateral view stout with a short tip, in caudal view pointed; and (4) parameres shorter than the aedeagus, in dorsal and lateral views with a dorsomesal tine. The male differs distinctly from that of *A. difformis* (McLachlan 1867) which exhibits the following combination of male genitalia characters: (1) lateral processes of segment X ("superior appendages") stout, wide; (2) median processes of segment X ("intermediate appendages") with tips projecting posterodorsad, posterior process of segment X in lateral view with 2 sharp projections, discernible in caudal and dorsal view as distinct, sharp tips; (3) general appearance of inferior appendages slender, in lateral view slender with a long tip, in caudal view rounded; (4) parameres longer than the aedeagus, lacking a dorsal tine.

Description. General appearance (Fig. 3A): yellow to fawn, tergites and sternites yellow to fawn; cephalic and thoracic setal areas cream-coloured; cephalic, thoracic and abdominal setation yellow; legs yellow to fawn; haustellum and intersegmental integument cream-coloured; wings yellow, translucent, with dark veins, setation on veins and membrane yellow. Male maxillary palps each trisegmented, spur formula 1,3,4.

Male genitalia (Figs. 4A–D). Tergite VIII yellow to fawn, with pair of suboval spinate areas clearly separated medially. Abdomen IX in lateral view lacking dorsad bulge. Lateral processes of segment X ("superior appendages") in lateral view slender, angled dorsad, in dorsal view posteriorly concave, in caudal view suboval, somewhat converging medially. Segment X clearly separated into pair of subtriangular vertical plates on either side of phallocrypt and between its lateral processes in caudal view, each with dorsal end bearing 1 long median process ("intermediate appendage") projecting nearly dorsad in lateral view; sclerites of segment X in lateral and caudal views each with rounded posterior process, this posterior process in dorsal view rounded subtriangular with sharp dorsal ridge. Inferior appendages partially fused with abdominal segment IX, in lateral view each stout with short, stout dorsal tip, this tip in caudal view pointed; in dorsal and caudal views inferior appendages stout. Aedeagus in lateral view distally bulbous, in dorsal view distal part semimembraneous. Parameres shorter than aedeagus, evenly curved dorsad; each with proximal part bulbous, bearing 2–3 ventral tines and distinct dorsomesal tine (best seen in dorsal view).

Female, pupa, and egg unknown.

Etymology. Named for Johann Waringer, Austrian entomologist.

Anisogamus difformis (McLachlan 1867)

Figs. 4E–H.

Material examined. 7 males; Austria, Carinthia, Saualpe, Ladinger Hütte, 28–29.vi.2012, leg. W. Graf. 7 males, 3 females: Italy, Torino, Fondo, Gias del Prete (45°31'13.37"N 007°39'40.59"E) 12.vii.2012, leg. G. Vinçon. 1 male: France, Provence Alpes-Côte d'Azur (44°20'16.4"N 006°47'27.9"E) 29.vii.2012, leg. W. Graf. 4 males: Austria, Carinthia, Saualpe, Offener Hütte, 30.vi.2006, leg. W. Graf. 9 males: Austria, Carinthia, Saualpe, Ladinger Alm, 29.vi.2012, leg. W. Graf. 27 males: Austria, Carinthia, Saualpe, Ladinger Hütte, 15.vii.2006, leg. W. Graf.

Type locality. Austria, Carinthia, Saualpe.

Description. General appearance (Fig. 3B) yellow to fawn, sclerites yellow to fawn; cephalic and thoracic setal areas pale cream-coloured; cephalic, thoracic and abdominal setation yellow; legs yellow to fawn; haustellum and intersegmental integument pale cream-coloured; wings yellow, translucent, with dark veins, setation on veins and membrane yellow. Male maxillary palps each trisegmented. Spur formula 1,3,4.

Male genitalia (Fig. 4E–H). Tergite VIII yellow to fawn, with pair of suboval spinate areas separated medially. Abdomen IX in lateral view with dorsal bulge (Fig. 4E, arrow). Lateral processes of segment X ("superior appendages") in lateral view stout dorsal lobes, in dorsal view posteriorly concave, in caudal view suboval, somewhat converging medially. Segment X clearly separated into pair of subtriangular vertical plates on either side of phallocrypt and between its lateral processes in caudal view, each with dorsal end bearing 1 median process ("intermediate appendage") projecting posterodorsad in lateral view; sclerites of segment X in lateral, dorsal and caudal views each with 2 pointed protuberances on rounded posterior processes, these posterior processes in dorsal view rounded subtriangular. Inferior appendages partially fused with abdominal segment IX, in lateral view slender with acute dorsal tip, this tip in caudal view rounded; in dorsal and caudal views inferior appendages slender, delicate. Aedeagus in lateral view distally bulbous, in dorsal view distal part semimembraneous. Parameres longer than aedeagus, evenly curved dorsad; each with proximal part bulbous, bearing 2–3 ventral tines.

Description of the larvae of the genus *Anisogamus*

Note: Photographs were obtained for larval specimens of *A. difformis* and *A. waringeri*. The description of larval characters refers to both species, if not stated otherwise. Larvae of *A. waringeri* were compared with larvae collected close to the type locality of *A. difformis* in Carinthia and morphological differences are described.

Larvae of the family Limnephilidae typically share the following set of morphological characters (Waringer & Graf 2011):

- sclerites present on pro-, meso-, and metanota;
- mesonotum completely covered by 2 sclerites, separated by unbranched longitudinal suture;
- metanotum incompletely sclerotized with 3 pairs of small sclerites in anteromedian, posteromedian and lateral positions [only 5 sclerites in *Hydatophylax* spp., metanotal sclerites reduced to just setal groups in some species];
- prosternal horn present [absent from *Drusus chrysotus* Rambur 1842];
- antennae situated halfway between eyes and anterior head margin;
- fleshy protuberances present laterally and dorsally on abdominal segment I.

In the genus *Anisogamus*, only lateral protuberances are present on abdominal segment I and the dorsum is completely covered by setae without a protuberance (Fig. 5A, triangle). To our current knowledge, this is the major character to distinguish larvae of the genus *Anisogamus* from all other limnephilid genera, which develop 3 protuberances on abdominal segment I. Ignoring the strikingly different morphology of abdominal segment I, recent larval determination keys focusing on Central European Chaetopterygini and Stenophylacini (Waringer & Graf 2011, 2013) key the larvae of *Anisogamus* spp. together with a group species in which larvae develop more than one posterolateral seta on each side of abdominal segment IX (*Allogamus ligonifer* McLachlan 1862, *Potamophylax* spp., *Platyphylax frauenfeldi* Brauer 1857, *Acrophylax zerberus* Brauer 1867 and *Psilopteryx psorosa* Kolenati 1860). By examining morphological characters listed by Waringer *et al.* (2013; table 1), *P. psorosa* remains the only taxon similar to *Anisogamus* spp. larvae. The ventral-edge setae on mid- & hind femora in *Anisogamus* have contrasting color, while all ventral-edge setae in *Psilopteryx* are dark (Fig. 5C, D).

Descriptions of the fifth instar larvae of *Anisogamus difformis/waringeri* sp. nov.

Material. *Anisogamus waringeri* **sp. nov.**: Four 5th instar larvae, France, Pyrenées orientales, Mont Canigou, Col de Jou, Refuge de Mariailles (42°29'6.21"N, 002°24'48.31"E), 13.vii.2013, leg. W. Graf. *Anisogamus difformis*: Five 5th instar larvae, Austria, Carinthia, Gleinalpe, Hochalm, 21.vi.2014, leg. W. Graf.

Biometry. *Anisogamus waringeri* **sp. nov.**: Body length of final instar larva (fixed in alcohol) 12.6–13.7 mm, head width 1.56–1.68 mm (n=3). *Anisogamus difformis*: Body length of final instar larvae (fixed in formaldehyde) 10–12.5 mm, head width 1.52–1.68 mm (n=5).

Head. Head capsule finely granulated with microspinules, slightly elongated in shape, hypognathous (Fig. 6A), with medium brown coloration. Muscle attachment spots on frontoclypeus and parietalia dark brown and distinct (Fig. 6A). Yellowish ring present around each eye (Fig. 6A). Head capsule with 17 pairs of primary setae, lacking seta #18 and any additional setation: Each parietal bearing 10 dorsal and 1 ventral primary setae, of which #9, 14, and 17 long and conspicuous. Further, in *A. difformis* lengths of setae #13 and 16 less than half as long as #15, whereas setae #13 and 16 more than half as long as #15 in *A. waringeri* **sp. nov.** Frontoclypeus bell-shaped with a narrow central constriction, each side bearing 6 primary setae, #5 long and conspicuous (Fig. 6B). Labrum medium brown; with setal brush and primary setae #1–3 at anterolateral margins; on dorsal area, setation consisting of primary setae #4–6. Antennae situated halfway between eyes and anterior head margin (Fig. 6A,B), short, each consisting of 1 short cylindrical base and 1 short flagellum. Ventral apotome narrow and elongate, medium brown, postgenal suture approximately 45–50% of apotome length. Each black mandible with 5 terminal teeth along its edge; in addition, ridges present in central concavity.

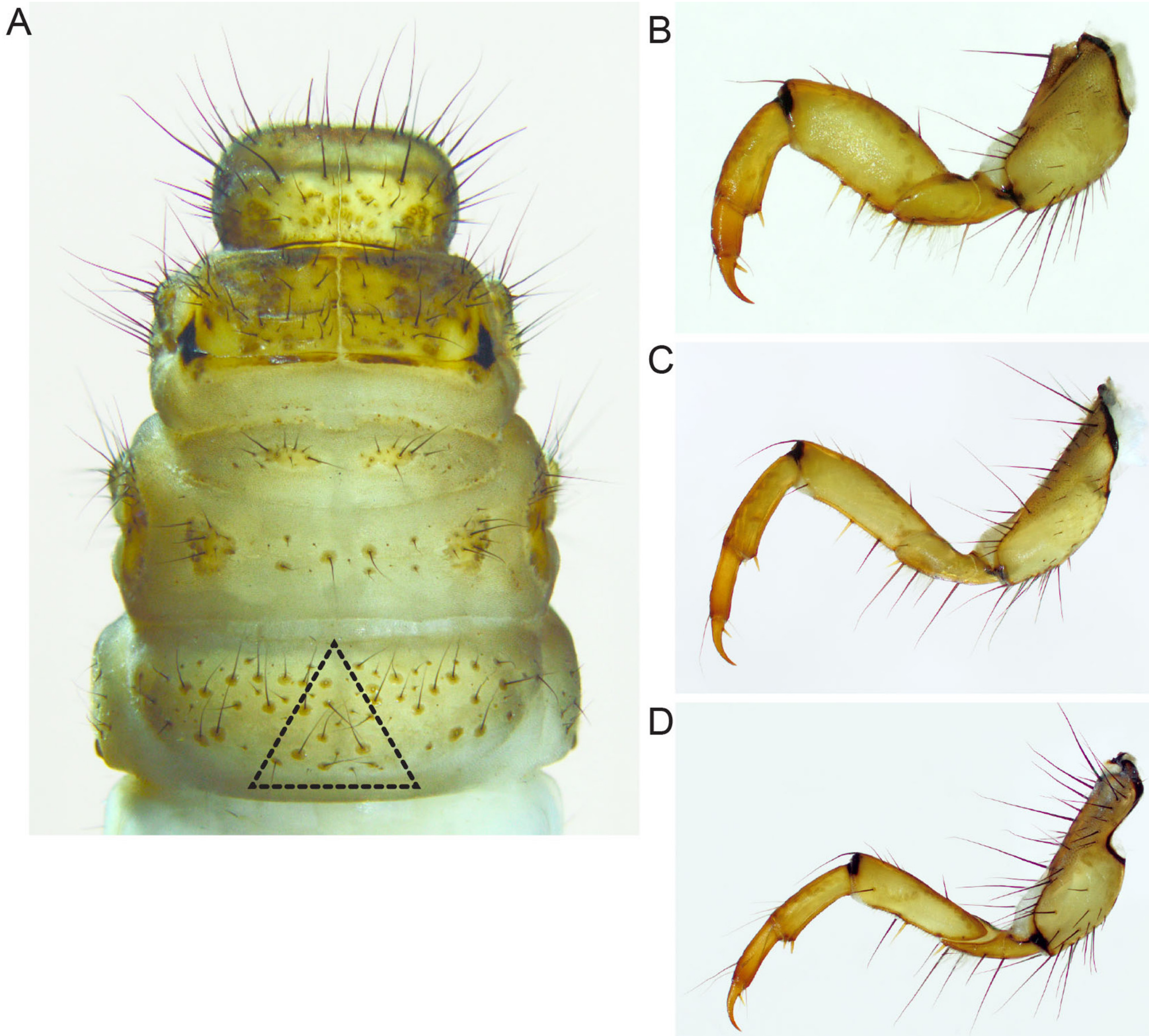

FIGURE 5. Larva of *A. waringeri* **sp. nov.** A, thorax and abdominal dorsum I, with the usual position of a dorsal protuberance marked with a dotted triangle; B, right prothoracic leg, anterior; C, right mesothoracic leg, anterior; D, right metathoracic leg, anterior.

Thorax. Pronotum medium to yellowish brown with dark brown muscle attachment spots and with finely granulate surface (Fig. 5A), its posterior and lateral margins thickened and darkly striped (Fig. 5A). Pronotal transverse groove at end of anterior 3rd (Figs. 6B, C, arrows); in profile, posterior 2/3rds very slightly rounded (Fig. 6C,D). Pronotal transverse groove much shallower in *A. waringeri* **sp. nov.** (Fig. 6D, arrow), more pronounced in *A. difformis* (Fig. 6C, arrow). Along anterior border three setal rows present: (1) dense fringe of short, curved, fine, yellow setae; (2) widely-spaced, continuous row of intermediate curved, pale yellow setae; and (3) widely-spaced, continuous row of long, straight, dark setae present. Following row (3), row of pale yellow (*A. difformis*) or dark (*A. waringeri* **sp. nov.**) setae present (Fig. 6E,F, arrows). Further, in *A. difformis*, dark and pale/ yellow setae present on pronotum, whereas in *A. waringeri* **sp. nov.**, only dark setae present. Including anterior setal rows (2) and (3), 40–50 setae of varying lengths distributed over each pronotal half in *A. waringeri* **sp. nov.**

(Fig. 7A), and 30–40 in *A. difformis*. Central prosternite inconspicuous; prosternal horn present. Mesonotum completely covered by 2 medium brown sclerites, their posterior margins darkly sclerotized (Fig. 5A). Metanotum partially covered by 3 pairs of medium brown sclerites. Anterior metanotal sclerites (sclerites of setal area 1, *sa*1) narrowly elliptical; distance between them greater than their length (Fig. 5A). Row of setae present between posteromedian sclerites (sclerites of setal area 2, *sa*2); setae lacking between each lateral (sclerites of setal area 3, *sa*3) and posteromedian sclerite (*sa*2) (Fig. 5A). Legs yellow to fawn with numerous setae on coxae, trochanters and femora; tibiae and tarsi with only small number of setae; all femora each with only 1 proximodorsal seta present (Figs. 5B–D). Coxa, femur and tibia of each foreleg much wider than those of mid- and hind legs. Additional setae lacking from anterior and posterior faces of all femora; ventral trochanteral brush at distal section of each trochanter present on all legs. Rows of minute spines present along ventral edges of femora; pairs of ventral-edge setae pale on forefemora, but pale and dark on mid- and hind femora, respectively (Figs. 5B–D).

Abdomen. Abdominal segment I with 2 lateral fleshy protuberances, lacking dorsal protuberance (Fig. 5A, triangle). Dorsal setal areas *sa*1 and *sa*2 fused, creating continuous band of setae completely spanning dorsum (Fig. 5A); setal area *sa*3 separate, small, covering dorsal section of each lateral protuberance (Fig. 7B, circle); 1 large, oval sclerite on posterior surface of lateral protuberance, lacking setae but bearing 2 holes (Fig. 7B). Ventral setal areas *sa*1 and *sa*2 fused to form continuous field of setae, many on basal sclerites of differing sizes, these sclerites sometimes fused in some specimens; setal areas *sa*1 not fused centrally leaving median bald patch (Fig. 7C, circle); setal areas *sa*3 separate, covering area below each lateral protuberance (Fig. 7B). On abdominal dorsum VIII, number of posterodorsal setae typically 8–16, with 4–6 long setae and 4–10 remaining setae short. Each half of abdominal dorsum IX with 1–3 posterolateral seta(e) (Fig. 7D, arrow). Further, pair of long stout setae present on abdominal venter IX (Fig. 7D, arrow). Median brown sclerite on abdominal tergum IX semicircular; along its posterior border, 7–8 long and several shorter setae present, 1 long seta having position of central intermediate *c* setae. Anal prolegs of limnephilid type, light brown, with medium brown muscle attachment spots. Anal claws medium brown, each with 1 small accessory hook. All gills single filaments. Dorsal gills present at most from segment II (presegmental position) to segment V (postsegmental position). Ventral gills ranging from segment II (presegmental position) to segment VII (postsegmental position). Lateral gills present from segment II (presegmental) to segment III (postsegmental position) in *A. waringeri* **sp. nov.**, lacking in *A. difformis*. Lateral fringe extending on each side from last 3rd of abdominal segment III to end of abdominal segment VIII, forked lamellae above lateral line absent.

Case (Fig. 7E). Larval case of mineral particles and leaf particles; length 13.1–14.8 mm in *A. waringeri* **sp. nov.** (n= 3), 11.7–12.9 mm in *A. difformis* (n=3); almost straight, slightly bend, tapering somewhat posteriorly (width at anterior opening 3.5–3.9 mm and at posterior opening 2.6–3.0 mm in both species).

Differential diagnosis of *Anisogamus* larvae. Larvae of *A. waringeri* **sp. nov.** and *A. difformis* differ quite distinctly in the following characters: (1) The pronotal transverse groove in lateral view is deep in *A. difformis*, shallow in *A. waringeri* **sp. nov.**; (2) the pronotal setation is dark and pale/yellow in *A. difformis*, only dark in *A. waringeri* **sp. nov.**; (3) lateral gills are lacking in *A. difformis*, present from segment II (presegmental) to segment III (postsegmental position) in *A. waringeri* **sp. nov.**

Anisogamus species delimitation and larval affiliation

An analysis of the genetic distance of mtCOI between two *Anisogamus* males clearly supports differentiation of *A. difformis* and *A. waringeri* **sp. nov.** as separate species. Uncorrected *p*-distances recorded in both fragments of the mtCOI gene (i.e., 8.2% and 9.6%, in the "barcode region" and the "S20-Jerry region", respectively; Table 1), are in line with interspecific distances commonly recorded in Limnephilidae (e.g., Graf *et al.* 2005, Previšić *et al.* submitted) and other caddisfly families (e.g., Hydropsychidae; Pauls *et al.* 2010). Haplotypes of the "barcode region" of the adult male of *A. waringeri* **sp. nov.** and the hitherto unknown larva collected at the same locality differed in a single base pair, whereas haplotypes of the "S20-Jerry region" were completely identical in these two specimens (Table 1). Since these values are well within the intraspecific variability of mtCOI usually observed in caddisflies (e.g., Pauls *et al.* 2009, 2010; Previšić *et al.* 2009, 2014) data at hand enable confident affiliation of the larva and the male of *A. waringeri* **sp. nov.**

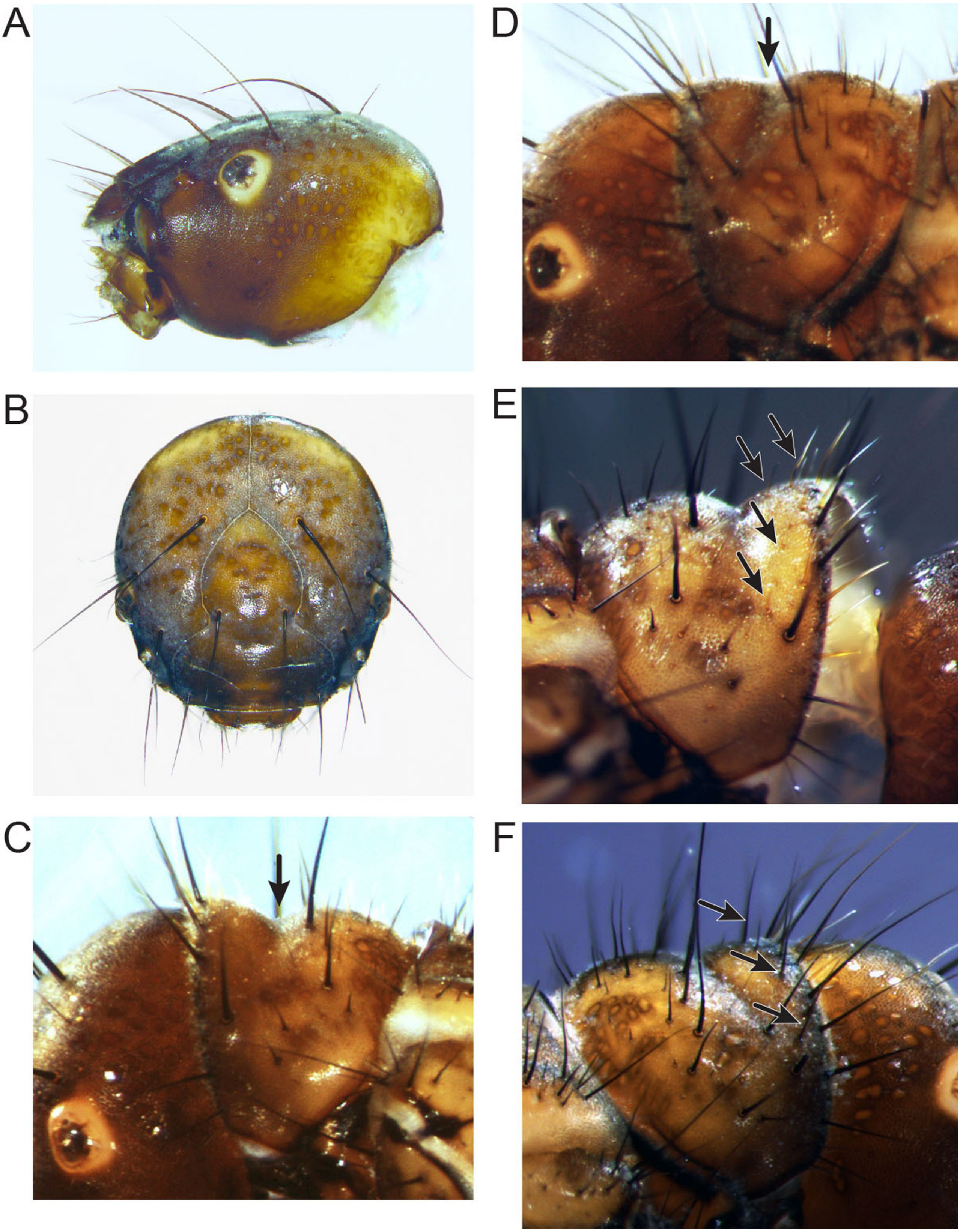

FIGURE 6. Larval characters of *A. waringeri* **sp. nov.** and *A. difformis*. A, *A. waringeri* **sp. nov.** head, left lateral; B, *A. waringeri* **sp. nov.** head, frontal; C, *A. difformis* pronotum, left lateral; D, *A. waringeri* **sp. nov.** pronotum, left lateral; E, *A. difformis* pronotum, right lateral (arrows indicating row of pale yellow setae); F, *A. waringeri* **sp. nov.** pronotum, right lateral (arrows indicating row of all-dark setae).

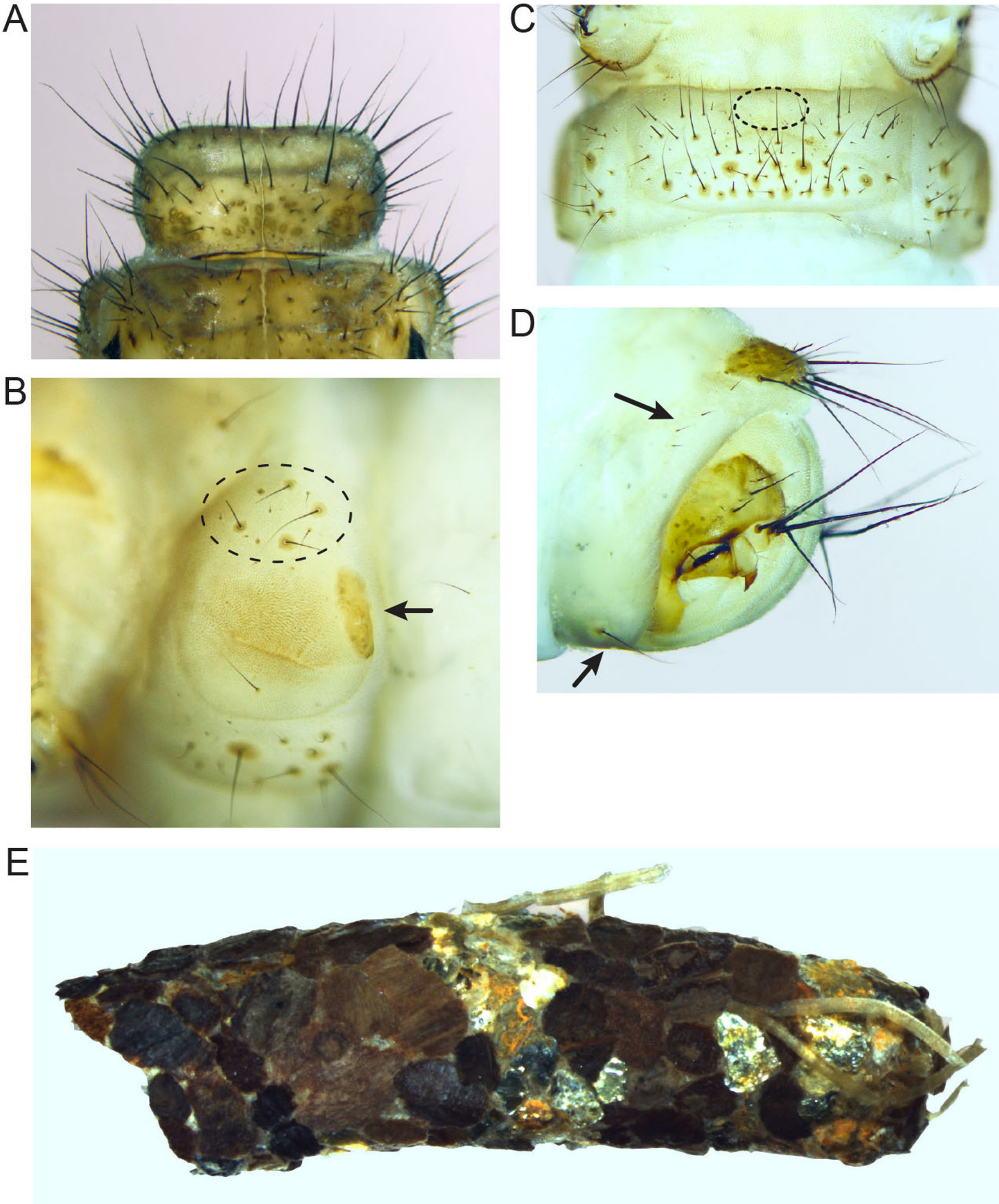

FIGURE 7. Larval characters of *A. waringeri* **sp. nov.** A, pronotum, dorsal; B, left lateral protuberance, left lateral (dotted circle indicating setal area *sa*3, arrow indicating associated sclerite); C, abdominal sternum I, with the separated setal areas 1 (*sa*1) marked with a dotted circle; D, left side of abdominal segments IX and X and left anal proleg and claw, left lateral (upper arrow indicating posterolateral setae of abdominal dorsum IX, lower arrow indicating long stout seta on abdominal venter IX); E. larval case of *A. waringeri* **sp. nov.**

TABLE 1. Inter- and intraspecific genetic distances of two mitochondrial cytochrome oxidase I (mtCOI) gene fragments recorded for *Anisogamus* species. Values below diagonal in second and third columns indicate number of nucleotide differences and above diagonal uncorrected pairwise distances (*p*) (shown as percents), respectively. Abbreviations are used to denote life stages; IM/M = adult male, L = larva.

Species	Stage	Specimen codes	mtCOI "barcode"		
			Andiff01	Ansp01	fAns0101L
Anisogamus difformis	IM/M	Andiff01		8.2	8.2
Anisogamus waringeri	IM/M	Ansp01	54		0.2
Anisogamus waringeri	L	fAns0101L	54	1	

TABLE 1. (Continued)

Species	mtCOI "S20-Jerry"			GenBank Access. No.	
	Andiff01	Ansp01	fAns0101L	"barcode"	"S20-Jerry"
Anisogamus difformis		9.6	9.6	KP174661	KP174658
Anisogamus waringeri	52		0	KP174662	KP174659
Anisogamus waringeri	52	0		KP174663	KP174660

Distribution & biogeography of *Anisogamus* species. The genus *Anisogamus* was established by McLachlan in 1874 based on the species *A. difformis*, and its type locality is situated in the Eastern Alps (Austria, Carinthia, Saualpe, Stelzing (Kimmins 1949)). Collated distribution data for *A. difformis* suggest a panalpine presence of the species (Fig. 3D).

Specimens of *A. waringeri* were collected at the Col de Jou, Mont Canigou, Pyrenées-Orientales, France. At a location close by, Décamps (1967) found putative *A. difformis* to be present (but very rare) in the valley of the Neste d'Aure at 1600 m a.s.l. and in the tributaries of the Têt river at 1100 m a.s.l. Specimens of *A. waringeri* **sp. nov.** were collected in the watershed of the Têt river, whereas the Neste d'Aure is some 125 km west of the recent collection points. Menéndez & González (2009) recorded *A. difformis* from the eastern Prepyrenees (Girona, Setcades), some 20 km south of the type locality of *A. waringeri* **sp. nov.**, and were re-identified by M. A. González as *A. waringeri* **sp. nov.** (pers. comm. M. A. González). From the same area, *Stenophylax nurianus* was described by Navás (1917), illustrating a specimen similar to the genus *Anisogamus*, but the type specimen is lost (pers. comm. M. A. González), and the description and the figure itself do not allow certain identification. Further, this species was proposed by Schmid (1949) to be a synonym of *A. difformis*, based on his own collection and identification of 2 putative *A. difformis* specimens. Thus, we consider *Stenophylax nurianus* a *nomen dubium* in concordance with Malicky (2005), justifying the description of *A. waringeri* **sp. nov.** We further conclude that *A. waringeri* **sp. nov.** is the single representative of the genus *Anisogamus* in the Pyrenees.

Acknowledgements

The authors are most grateful to Gilles Vinçon for his valuable material. Wolfgang Lechthaler, Vienna, provided outstanding photographs of larval characters. Füsün Sipahiler, Ankara, is thanked for valuable comments on the manuscript. WG acknowledges support of the BioFresh EU project-Biodiversity of Freshwater Ecosystems: Status, Trends, Pressures and Conservation Priorities (contract No. 226874); WG and SV acknowledge support of the Austrian Science Fund (FWF) (project number P23687-B17); AP acknowledges support of the University of Zagreb (PI: I. Ternjej, project number 202310). The authors thank Carmen Zamora-Muñoz, Marcos A. González, and John C. Morse for their vigilant reviews that significantly improved the quality of the manuscript.

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