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

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Abstract

New species are described from the following genera: *Conosorophylax* and *Anisogamus*, (Trichoptera, Limnephilidae). Additionally the larvae of the genus *Anisogamus*, and the larval stages of *Anisogamus waringeri* nov. sp. and *A. difformis* (McLachlan 1867) are described.

The new species *Conosorophylax vinconi* sp. nov. is a microendemic from the Southern Alps and differs distinctly 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*, *A. waringeri* sp. nov. develops more slender superior appendages; a more rounded basal plate of the intermediate appendages, lacking pointed protuberances; and parameres shorter than the aedeagus, 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 the genus *Anisogamus* are characterized by the lack of a dorsal protuberance on the 1st abdominal segment, a unique feature among Limnephilidae. *Anisogamus difformis* and *A. waringeri* sp. nov. larvae differ in pronotum shape.

The recovery 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 a biodiversity under threat of extinction that has yet to be fully described.

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Keywords

endemism; species description; *Conosorophylax*; *Anisogamus*; Europe; aquatic insects

Introduction

Both Alps and 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. (eds) 2003, Iserbyt et al. 2008, Huemer 2011). Increasingly, aquatic invertebrates (and EPT-taxa in particular) have become focus of attention in both Alps and Pyrenees (e.g., Sipahiler 1999, Sipahiler 2000, Graf 2005, Graf et al. 2008a, Malicky 2004, 2008, Brown et al. 2009). In the present paper, we describe two new species of caddisflies (Trichoptera) from the Pyrenees and the Alps.

The genus *Conosorophylax* 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).

Conosorophylax species show a complex distribution pattern, including several microendemics and two widespread species that inhabit the majority of the Alpine arc. Especially the Southern slopes of the Alps could be identified as centres of species richness in the genus, as the microendemics were exclusively found in the southern slopes of both 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 predominantly known from the Alps, but was also recorded in the Pyrenees. As the larva was hitherto not described, ecological parameters of adult collection points indicated a cold-stenotherm species 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 adult and larval specimens of putative *A. difformis* from the Pyrenees. 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 affiliate the larva of *A. waringeri* sp. nov.

In this paper, we describe the two new species and further provide a description of 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 analysis, respectively. Larvae of *A. difformis* were fixed and stored in formaldehyde for a prolonged period, impairing molecular analysis of those specimens.

Morphological characteristics of male terminalia were examined in KOH-treated, cleared specimens. Comparative material from the authors' collections enabled the identification of new species. Nomenclature of male terminalia follows Nielsen (1957). Larval morphological features were examined 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.

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* nov.sp. 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 Ltd., New Zealand) and aligned using MAFFT (Katoh & Standley 2007). Sequences were deposited in the GenBank under Accession nos: XXX (will be provided upon acceptance of the manuscript). 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

Vinçon's *Consorophylax*

Holotype—1 male: Italy, Torino, Valchiussella, Fondo, Burdeiver brook (45°30'59.60"N 7°39'09.72"E), 1800-1900m a.s.l., 01.09.2012, leg. G. Vinçon. Holotype deposited in the Linzer Landesmuseum. Paratype. 1 male, same collection dates, in coll. Malicky.

Type locality—Italy, Torino, Valchiussella, Fondo, Burdeiver brook at 1800-1900m a.s.l..

Diagnosis—A *Consorophylax* exhibiting (1) parameres that are distinctly constricted in the distal quarter; (2) inferior appendages that are slightly bifurcated in lateral view, with a sharp median tip in caudal view.

Description—General appearance light brown (in alcohol), tergites and sclerites light brown; cephalic and thoracic setal areas pale; cephalic, thoracic and abdominal setation light brown; legs light brown; haustellum and intersegmental integument pale, whitish; wings light brown, translucent, setation on veins and membrane light brown, forewing length 15 mm. Male maxillary palp trisegmented, tibial spur fomular 234.

Male terminalia (Fig. 1A-D). Dorsal third of segment IX reduced to a thin bridge, ventral 2/3rds broad, with a distinct triangular protuberance in lateral view. Tergite VIII light brown, with a median circular area of spines extending to a caudal ± bilobe area of spines. Superior

appendages (cerci *sensu* Snodgrass 1935) in lateral view roundish, with a proximal ventral bulge; in dorsal view $\pm$ triangular; in caudal view somewhat rectangular, medially concave. Intermediate appendages (paraproct *sensu* Snodgrass 1935) clearly separate, each bearing a 1 long mediodorsal tip, in lateral and dorsal view as a distinct caudad rod with a sharp dorsally curved tip; basal plate in lateral view with a median rounded bulge, in caudal view as separate $\pm$ triangular plate with median bulge and a dorsal projection. Inferior appendages (gonopods *sensu* Snodgrass 1935) in lateral view broad, stout with a caudadly dorsal portion, consisting of a median and a lateral tip; in dorsal view stout; in caudal view dorsal portion with a sharp median tip and a blunt lateral tip.

Aedeagus slender, in lateral view curved dorsally, in dorsal view tip bifurcate. Parameres in lateral view basally broad, abruptly constricted in the distal quarter to a slender, dorsally curved tip bearing 3-4 small spines.

Female & larva unknown.

Etymology—Named for the French entomologist Gilles Vinçon.

Distribution & biogeography of *Conсорophylax* species—The genus *Conсорophylax* 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, South-Eastern Alps), *C. piemontanus* Kimmins & Botosaneanu, 1967 (Piemont, Western Alps) (Fig. 2). *Conсорophylax styriacus* Kimmins & Botosaneanu, 1967 (Eastern Alps) and *C. consors* (McLachlan, 1880) (Western Alps) have a slightly larger distribution within the Alpine arc (Fig. 2). Although not knowing the exact distribution range *C. vinconi* sp. nov. is possibly another microendemic species within the genus. *Conсорophylax vinconi* sp. nov. is most similar to *C. piemontanus* Botosaneanu, 1967, which was described from Avigliana, some 13 km west of Torino but differs distinctly from this species. Particularly the inferior appendages and the parameres of the two species are of taxonomic importance. Interestingly, the type locality of *C. vinconi* sp. nov. is about 56 km north-north-east from the type locality of *C. piemontanus*. This supports the interpretation of *C. vinconi* sp. nov. as alpine microendemic.

***Anisogamus waringeri* sp. nov**

Graf & Vitecek

Waringer's *Anisogamus*

Holotype—1 male, France, Pyrénées-Orientales, Mont Canigou, Col de Jou, Refuge de Mariailles (42°29'6.21"N 2°24'48.31"E) 12.07.2012, leg. W. Graf. Holotype deposited at the Linzer Landesmuseum. Paratypes: 2 males, same location, 13.07.2012, 13.06.2013, leg. Graf.

Type locality—France, Pyrénées-Orientales, Mont Canigou.

Description—General appearance (Fig. 3A): yellow to fawn, tergites and sclerites yellow to fawn; cephalic and thoracic setal areas pale; cephalic, thoracic and abdominal setation yellow; legs yellow to fawn; haustellum and intersegmental integument pale, whitish; wings yellow, translucent, with dark veins, setation on veins and membrane yellow. Male maxillary palp trisegmented, spur formula 134.

Male terminalia (Fig. 4A-D). Tergite VIII yellow to fawn, with a medially clearly separated, $\pm$ oval spinate area. Abdomen IX in lateral view lacking a dorsad bulge. Superior appendages in lateral view a slender dorsad lobe, in dorsal view posteriadly concave, in caudal view $\pm$ oval, somewhat converging medially. Intermediate appendages clearly separate, each bearing 1 long median tip, projecting $\pm$ dorsal; basal plate in lateral, dorsal and caudal view with a rounded hump, basal plate in dorsal view $\pm$ triangular with a sharp ridge. Inferior appendages partially fused with abdominal segment IX, in lateral view stout with a short, stout dorsal tip, in caudal view pointed; in dorsal and caudal view stout. Aedeagus in lateral view distally bulbous, in dorsal view distal part $\pm$ membranous. Parameres shorter than the aedeagus, elegantly curved; proximal part bulbous, bearing 2-3 ventral tines, and a distinct dorsal tine (best seen in dorsal view).

Female unknown.

Etymology—Named for Johann Waringer, Austrian entomologist.

Anisogamus difformis (McLachlan 1867)

Material examined—7 males; Austria, Carinthia, St. Oswald, Saualpe, Ladinger Hütte, 28.-29.06.2012, leg. W. Graf.

Type locality—Austria, Carinthia, Saualpe.

Description—General appearance (Fig. 2A & C) yellow to fawn, tergites and sclerites yellow to fawn; cephalic and thoracic setal areas pale; cephalic, thoracic and abdominal setation yellow; legs yellow to fawn; haustellum and intersegmental integument pale, whitish; wings yellow, translucent, with dark veins, setation on veins and membrane yellow. Male maxillary palp trisegmented, spur formula 134.

Male terminalia (Fig. 3E-H). Tergite VIII yellow to fawn, with a medially separated, $\pm$ oval spinate area. Abdomen IX in lateral view with a dorsad bulge. Superior appendages in lateral view a stout dorsad lobe, in dorsal view posteriadly concave, in caudal view $\pm$ oval, somewhat converging medially. Intermediate appendages clearly separate, each bearing 1 long median tip, projecting posterodorsal; basal plate in lateral, dorsal and caudal view with 2 pointed protuberances, basal plate in dorsal view $\pm$ triangular. Inferior appendages partially fused with abdominal segment IX, in lateral view slender with an elegantly curved dorsal tip, in caudal view rounded; in dorsal and caudal view slender, delicate. Aedeagus in lateral view distally bulbous, in dorsal view distal part $\pm$ membranous. Parameres longer than the aedeagus, elegantly curved; proximal part bulbous, bearing 2-3 ventral tines.

Description of the larvae of the genus *Anisogamus*

Note: Photographs were obtained from specimens of *A. waringeri*. 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 of pro-, meso-, and metanota;
- mesonotum completely covered by 2 sclerites, separated by and unbranched longitudinal suture;
- metanotum incompletely sclerotized with 3 pairs of small sclerites in anteriomedian, posteromedian and lateral positions [only 5 sclerites in *Hydatophylax infumatus* McLachlan 1865, metanotal sclerites can be reduced to setal groups in some species];
- prosternal horn present [with the exception of *Drusus chrysotus* Rambur 1842];
- antennae situated halfway between eye and anterior head margin;
- fleshy protuberances present laterally and dorsally on the 1st abdominal segment.

In the genus *Anisogamus*, only lateral protuberances are present on the 1st abdominal segment, and the complete dorsum is medially covered by setae (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 the 1st abdominal segment. Ignoring the strikingly different morphology of the 1st abdominal segment, recent larval determination keys focusing on Central European Chaetopterygini and Stenophylacini (Waringer & Graf 2011, and Waringer et al. 2013) will key the larva of *Anisogamus* out together with a group species in which larvae develop more than one posterolateral setae on 9th abdominal segment (*Allogamus ligonifer* McLachlan 1862, *Potamophylax* spp., *Platyphylax frauenfeldi* Brauer 1857, *Acrophylax zerberus* Brauer 1867 and *Psilopteryx psorosa* Kolenati 1860). By examining morphological characters listed in Waringer et al. (2013; table 1) *P. psorosa* remains the only taxon similar to *Anisogamus* larvae. It can be easily separated by the contrasting colour of the ventral-edge setae on mid- & hind femora in *Anisogamus* while all ventral-edge setae in *Psilopteryx* are dark (Fig. 4B-D).

Description of the fifth instar larva of *Anisogamus difformis/waringeri* sp. nov

Material: *Anisogamus waringeri* sp. nov.: 4 5th instar larvae, France, Pyrénées orientales, Mont Canigou, Col de Jou, Refuge de Mariailles (42°29'6.21"N 2°24'48.31"E), 13.07.2013, leg. W. Graf. *Anisogamus difformis*: 5 5th instar larvae, Austria, Carinthia, Gleinalpe, Hochalm, 21.06.2014, leg. W. Graf.

Biometry: *Anisogamus waringeri* sp. nov.: body length of final instar larva (fixed in alcohol) 12.6 -13.7 mm, head width from 1.56 to 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* length of setae #13, 16 less than half the length of #15, whereas setae #13 and 16 are longer than half the length of #15 in *A. waringeri* sp. nov. Frontoclypeus bell-shaped with a narrow central constriction, each side bearing 6 primary setae, #5 long and conspicuous. Labrum medium brown 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), short, each consisting of 1 short cylindrical base and 1 short flagellum. Ventral apotome narrow and elongated, 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.

Thorax: Pronotum medium to yellowish brown with dark brown muscle attachment spots and with finely granulated surface (Fig. 5A), its posterior and lateral margins thickened and darkly striped (Fig. 5A). Pronotal transverse groove at end of anterior 3rd (Fig. 6B,C, arrows); in profile, posterior 2/3rds very slightly rounded (Fig. 6B,C). Pronotal transverse groove much shallower in *A. waringeri* sp. nov. (Fig. 6C, arrow), whereas it is more pronounced in *A. difformis* (Fig. 6B, arrow). Along anterior border three setal rows present: (1) dense fringe of short, curved, fine, yellow short setae; (2) widely-spaced, continuous row of intermediate curved, pale setae; and (3) widely-spaced, continuous row of long, straight, dark setae present. Following row (3), a row of pale (*A. difformis*) or dark (*A. waringeri* sp. nov.) setae is present. Further, in *A. difformis*, dark and pale/yellow setae are present on the pronotum, whereas in *A. waringeri* sp. nov., only dark setae are present. Including anterior setal rows (2) and (3), 40–50 setae of varying lengths distributed over each pronotal half in *A. waringeri* sp. nov., 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, sa1) narrowly elliptical; distance between them wider than their length (Fig. 5A). Row of setae present between posteromedian sclerites (sclerites of setal area 2, sa2); setae lacking between each lateral (sclerites of setal area 3, sa3) and posteromedian sclerite (sa2) (Fig. 4A). 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. 4B-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 fore femora, but pale and dark on mid and hind femora respectively (Figs. 5B-D).

Abdomen: First abdominal segment with 2 lateral fleshy protuberances, lacking a dorsal protuberance (Fig. 5A, triangle). Dorsal setal areas sa1 and sa2 fused, creating a continuous band of setae spanning over the whole dorsum (Fig. 5A); setal area sa3 separate, small, covering dorsal section of each lateral protuberance (Fig. 6F); 1 large, oval sclerite on lateral protuberance, lacking setae but bearing 2 holes (Fig. 6F). Ventral setal areas sa1 and sa2 fused to a continuous field of setae, many on basal sclerites of differing sizes, sometimes fused in some specimens; setal areas sa1 not fused centrally leaving a median bald patch (Fig. 6D, circle); setal areas sa3 separate, covering an area ventral the lateral protuberance (Fig. 6D). On 8th abdominal dorsum, number of posterodorsal setae typically 10–14, with 4–6 long setae and the 4 remaining setae short. Each half of 9th abdominal dorsum with 1–3 posterolateral seta(e) (Fig. 6E, arrow). Further, 2 long stout setae present on the 9th abdominal ventrum (Fig. 6E, arrow). Median brown sclerite on 9th abdominal tergum 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 2nd segment (presegmental position) to 5th segment (postsegmental position). Ventral gills ranging from 2nd segment (presegmental position) to 7th segment (postsegmental position). Lateral gills present from 2nd (presegmental) to 3rd segment (postsegmental position) in *A. waringeri* sp. nov., lacking in *A. difformis*. Lateral fringe extending from the last 3rd of the 3rd abdominal segment to the end of the 8th abdominal segment, forked lamellae dorsal the lateral line absent.

Case: Larval case of mineral particles and leaf particles; length 13.1–14.8 mm long 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* males—*Anisogamus waringeri* sp. nov. develops the following combination of male terminalia characters: (1) superior appendages slender, high; (2) intermediate appendages: median tip projecting dorsadly, basal plate in lateral view with 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, tip in caudal view pointed; (4) parameres shorter than the aedeagus, in dorsal and lateral view with a dorsal tine.

It differs distinctly from *A. difformis* (McLachlan 1867) which exhibits the following combination of male terminalia characters: (1) superior appendages stout, wide; (2) intermediate appendages: median tip projecting posteridorsad, basal plate in lateral view with a 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, tip in caudal view rounded; (4) parameres longer than the aedeagus, lacking a dorsal tine.

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

***Anisogamus* species delimitation and larval affiliation**

Analysis of 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, Previši et al. submitted) and other caddisfly families (e.g. Hydropsychidae; Pauls et al. 2010).

Haplotypes of the “barcode region” of 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 below the intraspecific variability of mtCOI usually observed in caddisflies (e.g. Pauls et al. 2009, 2010, Previši et al. 2009, 2014) data at hand enable confident affiliation of larvae and adults in *A. waringeri* sp. nov.

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 (Carinthia; Saualpe). Collated distribution data on *A. difformis* suggests a panalpine presence of the species (Fig. 3D).

Specimens of *A. waringeri* were collected at the Col de Jou, Mont Canigou, Pyrénées-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 1600m a.s.l. and in the tributaries of the Têt river at 1100m a.s.l. Specimens of *A. waringeri* sp. nov. were collected in 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.

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FIGURE 1.

Male terminalia and aedeagus of *Consorophylax vinconi* sp. nov. A, lateral view of the male terminalia; B, aedeagus in lateral and dorsal view; C, dorsal view of the male terminalia; D, caudal view of the male terminalia. In lateral view, the slightly bifurcated inferior appendages are visible. Further, the parameres are caudally constricted in lateral view (B, upper figure). Del. Vitecek.

FIGURE 2.

Known distribution of *Consorophylax* species. *Consorophylax consors* and *C. styriacus* are widely distributed in the Alpine region, whereas *C. carinthiacus*, *C. corvo*, *C. delmastroi*, *C. piemontanus* and *C. vinconi* sp. nov. are microendemics.

FIGURE 3.

Habitus and known distribution of *Anisogamus* species. A, *A. waringeri* sp. nov.; B, male *A. diffformis*; C, female *A. waringeri* sp. nov.; D, map of the known geographical distribution of *Anisogamus* species. Females of the genus are brachypterous, whereas males exhibit fully developed wings.

FIGURE 4.

Male terminalia and aedagi of *Anisogamus* species. A, lateral view in lateral (A,E), dorsal (C, G), and caudal (D, H) view. Additionally, the aedagi are illustrated in lateral and dorsal view. A-D, *A. waringeri*; E-H, *A. difformis*. Del. Vitecek.

FIGURE 5.

Larva of *A. waringeri* sp. nov. A, dorsal view of thorax and 1st abdominal dorsum lacking a dorsal protuberance (triangle); B, 1st thoracic leg in frontal view; C, 2nd thoracic leg in frontal view; D, 3rd thoracic leg in frontal view.

FIGURE 6.

Larval characters of *A. waringeri* sp. nov. and *A. difformis*. A, lateral view of the head capsule of *A. waringeri* sp. nov.; B, lateral view on the prothorax of *A. difformis*; C, lateral view on the prothorax of *A. waringeri* sp. nov.; D, lateral protuberance of *A. waringeri* sp. nov.; E, ventral view of the 1st abdominal sternum, showing the separated setal areas 1 (dotted circle); F, lateral view on the last segments of the abdomen of *A. waringeri* sp. nov.

Table 1

Inter- and intraspecific genetic distances of two mitochondrial cytochrome oxidase I (mtCOI) gene fragments recorded in *Anisogamus* species. Values below diagonal 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. (xxx accession nos will be provided upon acceptance of the manuscript).

Species	Stage	Specimen codes	mtCOI "barcode"			mtCOI "S20-Jerry"			GenBank Access. No.	
			Andiff01	Ansp01	fAns0101L	Andiff01	Ansp01	fAns0101L	"barcode"	"S20-Jerry"
<i>Anisogamus difformis</i>	IM/M	Andiff01		8.2	8.2		9.6	9.6	xxx	xxx
<i>Anisogamus waringeri</i>	IM/M	Ansp01	54		0.2	52		0	xxx	xxx
<i>Anisogamus waringeri</i>	L	fAns0101L	54	1		52	0		xxx	xxx