

Description of the exuvia and larva of *Pyrrhosoma elisabethae* (Odonata: Coenagrionidae)

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Abstract

During a field trip to the Greek island of Corfu which took place between 6 to 13 May 2012, a total of 348 exuviae and six larvae of *P. elisabethae* were collected for the first time. This quantity of *P. elisabethae* exuviae allows an accurate description of the species. The exuviae of *P. elisabethae* are very similar to the exuviae of *P. nymphula*, the principal differences being in the shape of their labial palps.

Zusammenfassung

Beschreibung der Exuvie und der Larve von *Pyrrhosoma elisabethae* (Odonata: Coenagrionidae) – Während einer Exkursion auf die griechische Insel Korfu zwischen dem 6. und dem 13. Mai 2012 wurden insgesamt 348 Exuvien und sechs Larven von *P. elisabethae* gesammelt. Die Menge der *P. elisabethae*-Exuvien erlaubt die präzise Beschreibung der Art. Die Exuvien von *P. elisabethae* sind denen von *P. nymphula* sehr ähnlich, die Hauptunterscheidungsmerkmale finden sich in der Gestalt der Labialpalpen.

Introduction

Pyrrhosoma elisabethae is one of the rarest and most vulnerable European damselflies (SAHLÉN et al. 2004; KALKMAN et al. 2010). The present distribution pattern of this species, confined to southern Albania and western Greece including Corfu, the Peloponnesus and the northwestern part of the mainland, overlaps only in northwestern Greece with that of *Pyrrhosoma nymphula* (BOUDOT et al. 2009; Lopau 2010). The adults of *P. elisabethae* were described by SCHMIDT (1948). Since then there has been confusion regarding the status of *P. elisabethae* and *P. nymphula* and proposals including “intermediate” or “subspecies” were used in BUCHHOLZ (1954), STARK (1979) and OTTOLENGHI (1991). In 2006, KALKMAN &

LOPAU published a morphological study that separates the adults of *P. elisabethae* and *P. nymphula* into two distinct species.

In 2012, C. Brochard and E. van der Ploeg undertook a trip to Corfu with its main aim being locating and collecting larvae and exuviae of *P. elisabethae*, which were required for the authors' joint project namely the description and photographic illustration of the exuviae of all European dragonflies. The field trip was successful and a short description of the larvae of the species was published in BROCHARD & VAN DER PLOEG (2013a, b).

The purpose of this paper is to describe the exuvia and the larva of *P. elisabethae* and, using morphological features, to compare the differences between the two closely related species *P. elisabethae* and *P. nymphula*. An identification key separating the two species is also provided.

Material and methods

Observations

During 6, 9 and 12 May 2012, on the Greek island of Corfu at the Melissoudi River, 1 km south-southeast of Sidari (39°46.603'N, 19°43.040'E, Fig. 1), *P. elisabethae* was located. For 10 days prior to 6 May, weather conditions were perfect and on the three days of collecting many individuals were observed actually emerging thus confirming the link with the exuviae. In total, 348 exuviae (Fig. 2) and 6 larvae were collected. In addition, 104 adults (Fig. 3, 4) were observed (BROCHARD & VAN DER PLOEG 2013a). The adults were identified as *P. elisabethae* using the criteria laid down in KALKMAN & LOPAU (2006) and DIJKSTRA & LEWINGTON (2008). No adults of *P. nymphula* were observed on the island.

Timing is a major factor in recording exuviae: searching too early in the season leads to finding only larvae and searching too late leads to observing only adults in univoltine species. Generally, the emergence period is difficult to predict and depends mainly on weather conditions (BROCHARD et al. 2012).

Material

The imagines were caught with a net and identified using a small hand loupe. The larvae were collected using a scoop net and were kept in a cool box during fieldwork. An aquarium and photographic equipment had been sent on ahead of the trip and were used to photograph the larvae alive. On account of the rarity of the species, only a few larvae were taken. Exuviae were found by locating suitable emergence sites. The collected exuviae were stored in small plastic boxes and put in a freezer for at least 12 hours in order to prevent damage by inadvertently trapped insects and spiders. Following freezing the plastic tubes were left open for several hours to ensure that the exuviae were completely dry and ready to be stored permanently.



Figure 1. Melissoudi River, habitat of *Pyrrhosoma elisabethae*, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 1: Fluss Melissoudi, Habitat von *Pyrrhosoma elisabethae*, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB



Figure 2. Exuviae of *Pyrrhosoma elisabethae*, Melissoudi River, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 2: Exuvien von *Pyrrhosoma elisabethae*, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB



Figure 3. Adult male of *Pyrrhosoma elisabethae*, Melissoudi River, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 3: Adultes Männchen von *Pyrrhosoma elisabethae*, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB



Figure 4. Immature female of *Pyrrhosoma elisabethae*, Melissoudi River, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 4: Frisches Weibchen von *Pyrrhosoma elisabethae*, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB

For detailed measurements two calibrated stereo microscopes equipped with micrometer eyepieces, Olympus SZ60 and Olympus SZ40, were used. Following selection of exuviae, pictures were taken using a Canon 5dMkII body mounted on several objectives such as Zeiss Luminar 25mm, Nikon CF-N 10x and 20x and Canon MP-E 65mm. Problems of depth of field were resolved using the stacking program Helicon Focus on an octa-core processor computer.

Methods of finding and selecting criteria

To define the key criteria a morphological study was first made by measuring the exuviae. These measurements are based on the exuviae and not on the larvae. Even though the exuviae are very fragile and difficult to handle, they present the full grown stadium of the larvae. Further, on account of the vulnerable status of *P. elisabethae*, the collection of exuviae made no impact upon the population. These morphological measurements, based on the exuviae of 25 males and 25 females (Tab. 2), give an overall view of the exuviae and a base for comparison with *P. nymphula*. The results of these measurements are used for the general description of the two species.

The exuviae and the larvae of *P. nymphula* have been described in several works (CONCI & NIELSEN 1956; ROBERT 1958; VELTHUIS 1960; DUIJM & DUTMER 1974; GEIJSKES & VAN TOL 1983; HAMMOND 1983; ASKEW 1988; CARCHINI 1993; NORLING & SAHLÉN 1997; GERKEN & STERNBERG 1999; HEIDEMANN & SEIDENBUSCH 2002; GRAND & BOUDOT 2006; BELLMANN 2007; CHAM 2009; DOUCET 2010; BROCHARD et al. 2012). Taking these references as a base, comparisons were made between the two *Pyrrhosoma* species. As mentioned above, all the exuviae of *P. elisabethae* were collected at the same location; however, *P. nymphula* is a common European species (DIJKSTRA & LEWINGTON 2008). In order to eliminate regional variations, exuviae of *P. nymphula* were examined from a number of different locations throughout Europe (Tab. 1).

Our strategy for identifying distinct identification criteria consists, when possible, of focussing on the most robust parts of the exuviae. For example, the caudal lamellae of *Pyrrhosoma* species are very fragile and often break during the emergence (BROCHARD et al. 2012). The prementum, however, is generally the strongest part of the exuvia (BROCHARD et al. 2012). In addition, parts of the exuvia that are difficult to measure such as head width – variable due to the width of the hole formed during emergence – and eye size were not taken into account. Finally, broken parts were not measured, as these could lead to inaccuracies.

Once a possible criterion had been identified, measurements were made to confirm or reject the supposition. The use of ratios can be useful as it allows the study of exuviae of varying sizes. Only those criteria that show no overlap in ratio between the two species were selected. To create and to validate the identification key, measurements were made based on 128 exuviae of *P. elisabethae* and on 156 exuviae of *P. nymphula*.

Table 1. Origins of *Pyrrhosoma nymphula* exuviae measured for the key. – Tabelle 1: Herkunft der für den Bestimmungsschlüssel vermessenen *Pyrrhosoma nymphula*-Exuvien.

Number of exuviae	Location & Country	collector
10	Groningen (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
10	Fochteloërveen (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
16	Diakonieveen (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
10	Balloërveld (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
9	Süderlügum (Germany)	C. BROCHARD & E. VAN DER PLOEG
8	Woldlakerbos (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
6	Zuidlaren (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
8	Appelbergen (the Netherlands)	C. BROCHARD & E. VAN DER PLOEG
12	Beaupréau (France)	B. GUILLON
10	Chatenay-Malabry (France)	M. JEANMOUGIN
10	Neuilly-Plaisance (France)	M. JEANMOUGIN
3	Lanildut (France)	M. JEANMOUGIN
7	Vicq-Exempt (France)	N. ROYER
2	Rajec (Slovakia)	D. ŠÁCHA
25	Sulzbach-Rosenberg (Germany)	R. SEIDENBUSCH
10	Viana do Castelo (Portugal)	A. SOARES
Total: 156		

Results

Description of the exuvia of *Pyrrhosoma elisabethae*

Habitus

The exuvia of *Pyrrhosoma elisabethae* is illustrated in Figure 5. The body length – excluding caudal lamellae – is about 12.5 mm and the caudal lamellae are about 4.7 mm long (Tab. 2). Males and females are very similar in size, not only for the body length, but also for many other body parts such as the length of prementum, antenna, posterior femur and distance between the scapi (Tab. 2).

The overall shape is slender, elongated and rather typical for a Coenagrionidae exuvia (BROCHARD et al. 2012). The colour varies from yellowish to dark brown.

Head

The head is broad in relation to the small body. Males and females have 7-segmented antennae (Tab. 2). The antennae are generally found in two colours, brownish and yellowish. The eyes are orientated sideways. The ocelli are not easily visible, especially in light coloured exuviae. The occipital corners are angular

Table 2. Measurements of 25 male and 25 female *Pyrrhosoma elisabethae* exuviae. – Tabelle 2: Maße der 25 männlichen und 25 weiblichen Exuvien von *Pyrrhosoma elisabethae*. Each measure with average (top), minimum and maximum value (bottom) and standard deviation in brackets; Messungen von Mittelwert (oben), dem Minimal- und Maximalwert (unten) sowie der Standardabweichung in Klammern.

	Total	Male	Female
Length excluding caudal lamellae	12.5 mm 11.7–13.5 mm (0.45)	12.4 mm 11.7–13.5 mm (0.48)	12.6 mm 11.8–13.3 mm (0.41)
Length caudal lamellae	4.7 mm 3.7–5.7 mm (0.31)	4.7 mm 3.7–5.7 mm (0.35)	4.8 mm 4.3–5.2 mm (0.26)
Length prementum	2.2 mm 2.1–2.4 mm (0.08)	2.2 mm 2.1–2.3 mm (0.06)	2.3 mm 2.2–2.4 mm (0.07)
Distal width prementum	2.1 mm 2–2.2 mm (0.05)	2.1 mm 2–2.2 mm (0.03)	2.1 mm 2.1–2.2 mm (0.04)
Basal width prementum	0.9 mm 0.8–0.9 mm (0.02)	0.8 mm 0.8–0.9 mm (0.02)	0.9 mm 0.8–0.9 mm (0.03)
Number setae of the left side of the prementum	3 2–3 (0.20)	3 2–3 (0.20)	3 2–3 (0.20)
Number setae of the right side of the prementum	3 2–3 (0.14)	3 2–3 (0.20)	3 3–3 (0.00)
Number setae on the left palpus	6.1 6–7 (0.30)	6.1 6–7 (0.33)	6.1 6–7 (0.28)
Number setae on the right palpus	6.1 6–7 (0.35)	6.2 6–7 (0.44)	6 6–7 (0.20)
Number antenna segment	7 7–7 (0.00)	7 7–7 (0.00)	7 7–7 (0.00)
Length antenna	2.1 mm 2.0–2.4 mm (0.08)	2.1 mm 2.0–2.4 mm (0.09)	2.1 mm 2.0–2.3 mm (0.08)
Length scapi	1.1 mm 1.0–1.1 mm (0.03)	1.1 mm 1.0–1.1 mm (0.02)	1.1 mm 1–1.1 mm (0.02)
Length hindwing case	4.2 mm 3.9–4.6 mm (0.17)	4.1 mm 3.9–4.3 mm (0.14)	4.3 mm 4.1–4.6 mm (0.15)
Length forewing	4.5 mm 4.2–4.9 mm (0.16)	4.4 mm 4.2–4.6 mm (0.11)	4.6 mm 4.4–4.9 mm (0.13)
Length posterior femur	3.4 3.2–3.6 mm (0.12)	3.3 3.2–3.5 mm (0.09)	3.5 3.3–3.6 mm (0.12)





Figure 5. *Pyrrhosoma elisabethae* exuviae. – Abbildung 5: *Pyrrhosoma elisabethae*-Exuvien. **a** male dorsal view, Männchen, Rückenansicht; **b** male ventral view, Männchen, Bauchansicht; **c** male lateral view, Männchen, Seitenansicht; **d** light male dorsal view, hell gefärbtes Männchen, Rückenansicht; **e** dark male dorsal view, dunkel gefärbtes Männchen, Rückenansicht; **f** prementum dorsal view, Praementum, Rückenansicht; **g** prementum ventral view, Praementum, Bauchansicht; **h** right labial palp, rechter Labialpalpus; **i** prementum fascial view, Praementum, Vorderansicht; **j** caudal lamellae showing dark pattern, Kiemenblättchen mit dunkler Zeichnung; **k** caudal lamellae showing light pattern, Kiemenblättchen mit heller Zeichnung. Photos: CB

and spiny. The prementum is triangular shaped with, on the inside, two diagonal rows of generally three setae. Each labial palp has generally six setae (Tab. 2). Furthermore, the number of premental setae and palpal setae was found to be the same in males and females. The distal comb of the labial palp is slender with blunt teeth. The end hook of the labial palp is slightly curved and gradually widening.

Thorax

The legs have generally two dark and three light rings on the femora and the tibiae have generally one dark and two light rings. The wing cases are generally of the same colour as the abdomen.

Abdomen

The abdomen is elongated and cylindrical, gradually tapering caudally. A light mid-dorsal band is present on most exuviae. The whole surface is covered with small, strong hairs. The carina lateralis is lighter than the sternites and the tergites. Sternites 8 and 9 are without setulation on the hind border.

Caudal lamellae

Caudal lamellae have the two paraprocts and the epiproct of similar shape and size. The overall shape is elongated, rounded at the distal part and often ending in a small point. The lamellae are light brown at the base, becoming darker brown to almost black at the distal part. The dark patterning is often x-shaped. There is a row of setae on the underside of the paraprocts, approximately one third of the distance from the base. This setulation is absent from the upper surface of the paraprocts and barely visible on either side of the epiproct. The nodal line, characteristic in many species of Coenagrionidae including *Coenagrion* and *Enallagma* (BROCHARD et al. 2012), is barely visible but can be detected crossing the caudal lamellae with a curve two thirds from the base. The surface of the caudal lamellae is marked with light dots, especially visible on the dark parts.

Discussion

The separation of *Pyrrhosoma* exuviae within the family Coenagrionidae

The key proposed in BROCHARD et al. (2012) can be used to separate the genus *Pyrrhosoma* from other European Zygoptera.

Pyrrhosoma and *Ceriagrion* differ from the other Coenagrionidae by the angled corner of the occiput which is rounded in the other genera of this family. In addition, the exuviae of *Pyrrhosoma* and *Ceriagrion* are not transparent like the ones of most of the coenagrionid species (BROCHARD et al. 2012). The main difference between *Pyrrhosoma* and *Ceriagrion* is that in *Ceriagrion* the hind borders of segments 8 and 9 have small, but strong spines and in *Pyrrhosoma* the hind borders of segments 8 and 9 lack spines (BROCHARD et al. 2012).

Comparisons between exuviae of *P. elisabethae* and *P. nymphula*

As is the case with the adult insects, the exuviae of *P. elisabethae* and *P. nymphula* are very similar. Two separating criteria are proposed in this key both of which relate to the prementum. The first is based on measurements leading to a ratio on which the identification is based. The second criterion describes a visible difference best achieved when both species are examined together (Fig. 6, 7). Therefore, criterion 1 is considered more reliable when only one of the species is under examination.

- 1 **Criterion 1** – inner length of comb (a) / width of comb (b) < 1.9 (Fig. 6)
Criterion 2 – palpal hook less curved and slender widening (c, Fig. 6)
 (n=128, range inner length of comb / width of comb: 1.00-1.75)
 -> *P. elisabethae*
- 1' **Criterion 1** – inner length of comb (a) / width of comb (b) > 1.9 (Fig. 7)
Criterion 2 – palpal hook acutely widening, angular or even slightly notched (c, Fig. 7) (n=156, range inner length of comb / width of comb: 2.00-4.50) -> *P. nymphula*

These two criteria were chosen because no overlap in measurements or shape was observed between the two species.



Figure 6. Labial palp of *Pyrrhosoma elisabethae* exuviae. – Abbildung 6: Labialpalpus einer Exuvie von *Pyrrhosoma elisabethae*. **a** inner length of comb, Innenlänge des Kamms; **b** width of comb, Breite des Kamms; **c** palpal hook, Endhaken des Labialpalpus. Photos: CB



Figure 7. Labial palp of *Pyrrhosoma nymphula* exuviae. – Abbildung 7: Labialpalpus einer Exuvie von *Pyrrhosoma nymphula*. **a** inner length of comb, Innenlänge des Kamms; **b** width of comb, Breite des Kamms; **c** palpal hook, Endhaken des Labialpalpus. Photos: CB

In general the prementum of *P. nymphula* is broader than in *P. elisabethae*. The caudal lamellae are also different, higher in lateral view in *P. nymphula* and more slender in *P. elisabethae*. However, both species show considerable variation, therefore differences in colour or the shape of caudal lamellae are not reliable criteria.

Additional information about the larvae

According to the exuviae, the larvae of *P. elisabethae* (Fig. 8, 9) and *P. nymphula* are very similar (BROCHARD & VAN DER PLOEG 2013b). Both species show the same colour variation from yellowish to dark brown and have the same slender body with broad head. The eyes of *P. elisabethae* (Fig. 10) are, as in *P. nymphula*, crossed with two dark bands. These dark bands are not visible on the exuviae. The carina lateralis is lighter than the rest of the abdomen, even in light brown larvae. The light mid-dorsal band is, as in the exuviae, clearly visible on the larvae.

The larvae of *P. elisabethae* were found in sluggish streams, with an average depth of 1.5 m and an average width of about seven meters during summer. In autumn and winter, these streams can have very fast currents due to heavy rainfall, reach-



Figure 8. Female larva of *Pyrrhosoma elisabethae* in lateral view, Melissoudi River, south-east of Sidari, Corfu, Greece (6-v-2012). – Abbildung 8: Weibliche Larve von *Pyrrhosoma elisabethae* in Seitenansicht, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB

ing a depth of almost 10 m. The larvae live primarily in lentic parts of the rivers partly shaded by rich vegetation. The larvae can be found between the roots of riparian bushes and trees (Fig. 11).

The larvae emerge close to the river margin in the nearby vegetation. Most of the exuviae were found within a distance of 3 m from the margin, mainly close to ground level; however, some exuviae were collected up to 1.8 m above the ground. Like many other species of dragonflies, the emergence period depends on weather conditions (BROCHARD et al. 2012). During the three days of fieldwork the larvae were emerging. However, it is fair to assume that the emergence period commenced at least a week earlier as copulae and egg-laying females were observed at the same time.

Further studies

This study is based only on exuviae of *P. elisabethae* collected on the island of Corfu. In order to improve the key, it would be interesting to study material from the Peloponnese. Also a study of exuviae from *P. elisabethae* and *P. nymphula* is needed in the overlap zone of the Greek-Albanian borderland as well as in other eastern European countries for *P. nymphula*.



Figure 9. Female larva of *Pyrrhosoma elisabethae* in dorsal view, Melissoudi River, south-east of Sidari, Corfu, Greece (6-v-2012). – Abbildung 9: Weibliche Larve von *Pyrrhosoma elisabethae* in Rückenansicht, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB



Figure 10. Female larva of *Pyrrhosoma elisabethae* in frontal view, Melissoudi River, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 10: Weibliche Larve von *Pyrrhosoma elisabethae* in Vorderansicht, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB



Figure 11. Larval habitat of *Pyrrhosoma elisabethae*, Melissoudi River, southeast of Sidari, Corfu, Greece (6-v-2012). – Abbildung 11: Larvalhabitat von *Pyrrhosoma elisabethae*, Fluss Melissoudi, südöstlich von Sidari, Korfu, Griechenland (06.05.2012). Photo: CB

The genus *Pyrrhosoma* has been the subject of recent studies on molecular phylogeny (DIJKSTRA et al. 2013; GUAN et al. 2013). One of the conclusions in GUAN et al. (2013) is that specimens of *P. nymphula* from Morocco are phylogenetically very similar to *P. elisabethae*. It would, therefore, be interesting to study *P. nymphula* exuviae from Morocco in order to compare them with those of *P. elisabethae*.

The study of odonate larvae provides important information on the habitat selection of Odonata (BROCHARD et al. 2012). More research on the microhabitat of *P. elisabethae* larvae in other locations would supply a more detailed picture of the habitat requirements of this vulnerable species, which could be used as a basis for habitat management.

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