

## ADDITIONAL RECORDS AND A NEW DESCRIPTION OF *PROTONEMURA KHROUMIRIENSIS* SP. N. (PLECOPTERA, NEMOURIDAE) FROM TUNISIA

*Mustapha BÉJAOUÏ & Moncef BOUMAÏZA*

Unité d'Hydrobiologie Littorale et Limnique. Laboratoire de Biosurveillance de l'Environnement.

Faculté des Sciences de Bizerte. 7021 Jarzouna, Tunisia

E-mail: bejaouimustapha@yahoo.com

### ABSTRACT

*Protonemura khroumiriensis* sp.n. has been recorded in several valleys in the Tunisian Khroumirian mountains. The species is morphologically closely related to *Protonemura ruffoi* from the *Protonemura corsicana* group, which has a Circum-Mediterranean and Caucasian distribution. Particularly the male is well distinguished by the shape of its epiproct, lack of apical appendix of the epiproct, the shape of the paraprocts and vesicle on sternite 9. The female can be distinguished by its trapezoidal subgenital plate. The subgenital plate is well sclerotized, except in the middle area. This species preferentially colonizes temporary streams and adapts its life cycle to their flowing periods. Its flight period lasts from late winter to late spring, but differs slightly from site to site.

**Key words:** Nemouridae, *Protonemura khroumiriensis*, Khroumirian valley, Tunisia, new species

## NUOVE SEGNALAZIONI E DESCRIZIONE DI *PROTONEMURA KHROUMIRIENSIS* SP. N. (PLECOPTERA, NEMOURIDAE) IN TUNISIA

### SINTESI

*Protonemura khroumiriensis* sp.n. è stata segnalata in diverse valli delle montagne tunisine di Khroumirian. La specie è morfologicamente strettamente legata a *Protonemura ruffoi* del gruppo *Protonemura corsicana*, che ha una distribuzione circum-mediterranea e caucasica. In particolare, il maschio si distingue bene per la forma dell'epiprocto, l'assenza dell'appendice apicale dell'epiprocto, la forma del paraprocto e la vescicola sul 9° sternite. La femmina viene distinta per la forma trapezoidale della placca subanale. Tale placca è ben sclerificata, tranne che nella parte centrale. La specie preferisce colonizzare temporaneamente corsi d'acqua ed adatta il proprio ciclo di vita ai periodi di piena. Il periodo di volo della specie va dal tardo inverno alla tarda primavera, ma può variare da sito a sito.

**Parole chiave:** Nemouridae, *Protonemura khroumiriensis*, valle di Khroumirian, Tunisia, nuova specie

## INTRODUCTION

Stoneflies constitute a very important component of the freshwater macroinvertebrate communities. The larvae are found almost exclusively in running waters and reach their greatest diversity in small and high altitude streams. They are generally associated with coarse substrate such as cobble, leaf packs and woody debris. Plecoptera such as other orders of aquatic insects is very sensitive to the changes in water components, such as dissolved oxygen dose, mineralization of water, and pollution risks. This explains why species belonging to this order are considered good bioindicator of freshwater ecosystems. Plecoptera is one of the key taxa in many bio-index methods adopted in such studies (Alba-Tercedor & Sánchez-Ortega, 1988; Alba-Tercedor *et al.*, 2002). Stonefly adults have a very short life (1–4 weeks), compared with the length of their immature stage (6 months to 3 years) (Bouchard, 2004). Plecoptera species can be distinguished by several characteristics, but mostly by the morphology of their genitalia: epiproct and paraproct of the male and, from Nemouridae, subgenital plate of the female.

Berthélemy (1973) provided the first list of Tunisian stoneflies, including 17 species collected in valleys of Khroumiry (North-western Tunisia). Further, of the 5 *Leuctra* species recorded in the region, 4 were described as new species by Pardo & Zwick (1993) and Vinçon & Pardo (1998). *Amphinemura chiffensis* (Aubert, 1956) was recorded in Khroumiry and its nymph described for the first time (Béjaoui *et al.*, 2002–2003; Béjaoui & Boumaïza, 2004). *Protonemura algirica* (Aubert, 1956) was found by Boumaïza (2002). Additionally, two new taxa of genus *Protonemura*, *Protonemura drahamensis*

and *P. algirica bejaiana* were described by Vinçon & Pardo (2006) and Vinçon & Murányi (2009), respectively.

Four other species of genus *Protonemura* were reported from northern Africa: *P. tyrrhena* (Festa, 1938), *P. talboti* (Navás, 1929), *P. ruffoi* (Consiglio, 1961), *P. berberica* (Vinçon & Sánchez-Ortega, 1999), and *P. dakkii* (Vinçon & Murányi 2009 (Aubert, 1952, 1956; Berthélemy, 1973; Gagneur & Aliane, 1991; Vinçon & Sánchez-Ortega, 1999).

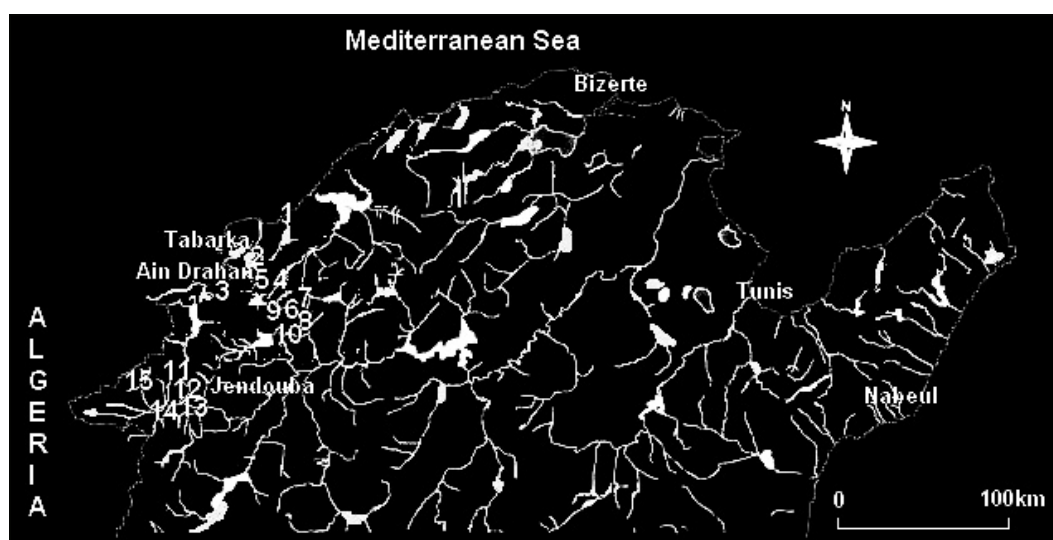
The phenology of the species involves seasonal time of the life cycle's processes and synchronization of these processes. In Tunisia, the phenology of the Plecoptera species was studied for the first time by Béjaoui (2004).

During samplings in several Khroumiry mountain valleys we caught a number of specimens (adult and larvae) belonging to the new *Protonemura* species described below.

## MATERIAL AND METHODS

## Study area

The specimens of this species were collected in several mountain valleys located in North Western Tunisia close to Algerian border (Fig. 1). This region is the most humid in Tunisia, receiving more than 1200 mm per year of rain at an altitude ranging from 500 to 1200 m a.s.l. The streams are generally small and surrounded by dense vegetation, consisting predominantly of *Quercus faginea* and *Q. suber*. The water is cold, with low salinity and conductivity, and with high content of dissolved oxygen (Tab. 1).



**Fig. 1: Location of *Protonemura khroumiriensis* sampling sites.**  
**Sl. 1: Lokacije vzorčnih mest za *Protonemura khroumiriensis*.**

**Tab. 1: Mean values of the basic physico-chemical parameters of the water: AL = altitude (m); WD = width (m); DP = depth (cm); VC = velocity current ( $\text{cm s}^{-1}$ ); AT = air temperature ( $^{\circ}\text{C}$ ); WT = water temperature ( $^{\circ}\text{C}$ ); EC = conductivity ( $\mu\text{s cm}^{-1}$  at  $20^{\circ}\text{C}$ ); S = salinity (psu); DO: dissolved oxygen ( $\text{mg l}^{-1}$ ); pH.**

**Tab. 1: Srednje vrednosti osnovnih fiziokemičnih parametrov vode: AL = nadmorska višina (m); WD = širina (m); DP = globina (cm); VC = hitrost toka ( $\text{cm s}^{-1}$ ); AT = temperatura zraka ( $^{\circ}\text{C}$ ); WT = temperatura vode ( $^{\circ}\text{C}$ ); EC = prevodnost ( $\mu\text{s cm}^{-1}$  pri  $20^{\circ}\text{C}$ ); S = slanost (psu); DO = raztopljeni kisik ( $\text{mg l}^{-1}$ ); pH.**

Sites and its number on Fig.1	AL	WD	DP	VC	AT	WT	EC	S	DO	pH
1-Wadi Bouterfes	25	1.8	17.5	13.7	20.5	15.1	1157.9	0.3	10.5	8.03
2-Wadi Rennagha	10	1.5	17.5	17.1	19.3	16	1013	0.4	10.5	8.19
3-Wadi Ennour	400	1.5	20	16.6	17.3	16.2	528.8	0.2	11	8.25
4-Wadi Lasfar	520	4.5	12.5	26.2	17.3	12.2	176.8	0.2	10.4	6.75
5-Wadi Bransia	630	2.3	7.5	28	17.4	10.8	164.1	0.1	11.7	6.25
6-Wadi EdDmène (1)	750	0.25	12.5	13.3	15	9	130.4	0.1	11.7	6.25
7-Wadi Sardouk	625	0.35	7.5	16	17.5	9	171.1	0.1	10.6	6.25
8-Châabet Kef El Ouachi	615	1.75	12.5	12.3	17.7	11	175	0.1	10.4	6.32
9-Wadi EdDmène (2)	570	2.5	15	15.2	17.7	12.2	227	0.2	10.5	6.75
10-Châabet El Magroun (Fernana)	245	0.8	25	14.4	21.1	15.9	925.4	0.4	10	8.14
11-Wadi M'zaïef	630	0.4	12.5	12.8	17	12.2	162.7	0.1	11.3	6.75
12-Châabet El Magrouna (Aïn Soltane)	570	0.5	12.5	14.5	16.9	12.3	165.1	0.1	11.3	6.25
13-Wadi EsSoufi	230	1.2	15	11.3	18	14.3	214.3	0.2	10.1	6.75
14-Wadi Hadjar	220	1.3	15	11.2	18.9	14.5	207.9	0.2	10.1	6.25
15-Wadi Mouadjen	760	0.9	12.5	16.3	14.1	12.2	124.9	0.1	11.9	6.25

### Sampling, conservation and studying methods

The adults of *P. khroumiriensis* were collected with entomological pliers placed over the stones or vegetation debris bordering the streams. The larvae were collected with the Surber net (Surber, 1937). The specimens were conserved in 70% ethanol. Description of their external morphology and illustrations of these forms were made, using a binocular stereomicroscope CARL ZEISS (0.63-6.3X), provided with a lateral drawing tube. Photographs were taken with a numerical camera, OLYMPUS (Model-C) with Zoom assembled on a binocular stereomicroscope OLYMPUS (Model SZ1145TR).

## RESULTS AND DISCUSSION

### Adult material studied

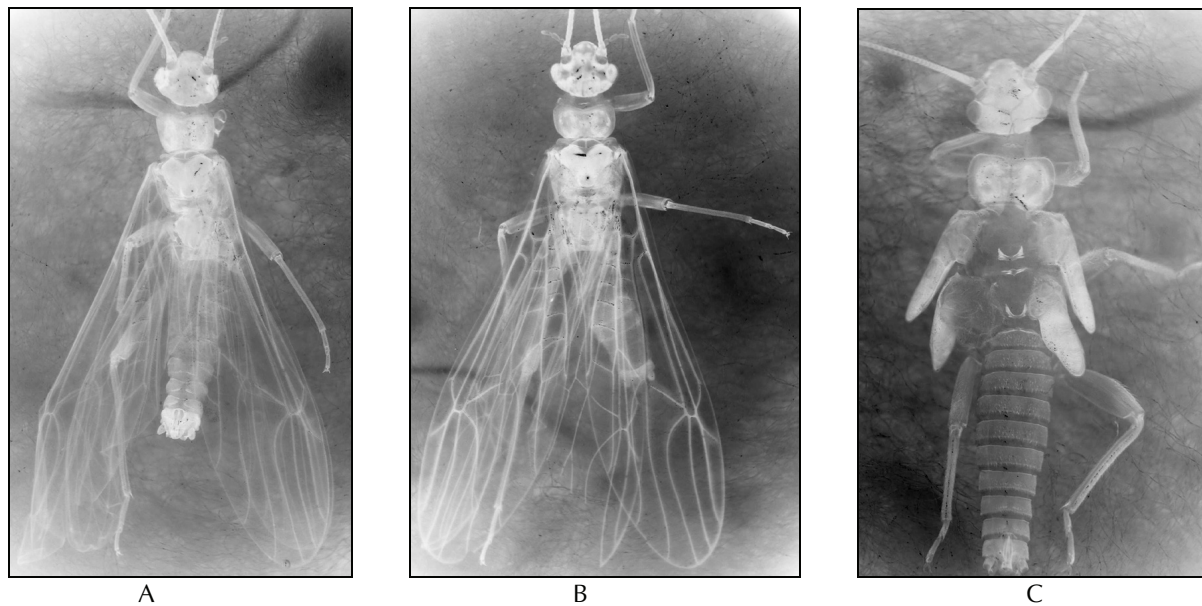
TUNISIA: Valley Ennour, 400m; 18-III-98: 1♂ (Holotype), 12-III-99: 9♂♂+6♀♀ (Paratypes), 8-IV-99: 3♀♀, 9-V-99: 1♀. Valley Lasfar, 520m; 18-III-98: 1♀. Valley Bransia, 630m; 18-III-98: 2♂♂+3♀♀, 8-IV-99: 5♂♂+ 4♀♀. Valley EdDmène, 750m; 24-III-99: 2♀♀, 6-V-99: 2♂♂+2♀♀. Valley EdDmène, 570m; 24-III-99: 1♂+6♀♀, 6-V-99: 11♂♂+19♀♀, 30-V-99: 3♀♀. Valley Sardouk, 625m; 24-III-99: 7♂♂+2♀♀, 6-V-99: 2♂♂. Châabet Kef El Ouachi, 615m; 24-III-99: 11♂♂+24♀♀, 6-V-99: 2♀♀. M'zaïef valley, 630m; 9-IV-2000: 1♂+1♀. Châabet El Magrouna, 570m; 9-IV-2000: 2♂♂+1♀. Valley Mouadjen, 760m; 9-IV-2000: 1♀. The holotype

and paratypes are deposited in the Laboratoire d'Hydrobiologie, Faculté des Sciences de Bizerte, Université 7 novembre à Carthage (Tunisia).

### General diagnosis of the adults

Mean body length: male 7.1 mm, female 6.7 mm. Mean forewing length: male 9.4 mm, female 9.5 mm. General colour brown; head dark brown; legs light brown; antennae blackish with brownish spots at their insertion (Figs. 2A, B). Pronotum sub-rectangular and blackish (Fig. 3A). Gills simple without apical constriction.

**Male diagnosis** (Figs. 3B-F). Tergites 7, 8 and 9 slightly sclerotized, with groups of spines on central posterior margins. Tergites 8 and 9 with two median lobed expansions (resembling those of *P. ruffoi* and *P. tyrrhena*). 10<sup>th</sup> tergite with short spines around epiproct tip, visible in lateral view. Sternite 9 distally extended into a subgenital plate (hypoproct), basally with a long racket-shaped and well developed vesicle (2-2.5 longer than wider) exceeding posterior margin of 9<sup>th</sup> urite. Epiproct flattened and sarcophagus shaped in dorsal view; without small apical appendix (this is a character that distinguishes this species well from other North African *Protonemura* species). In lateral view, epiproct wide and narrowed near the tip, slightly curved upward. Ventral sclerite flat, with short spines. Paraprocts (terminology of Baumann, 1975): inner lobes hidden by the subgenital plate; median lobes with a wide basal sclerotized por-



**Fig. 2:** *P. khroumiriensis* sp. n. Dorsal view of (A) adult male, (B) adult female and (C) mature larva.

**Sl. 2:** *P. khroumiriensis* sp. n. Dorzalni pogled na (A) odraslega samca, (B) odraslo samico in (C) ličinko v zreli fazi.

tion, inner darkly sclerotized branch of the median lobe curved, thick, long and sharp end, without a group of apical spines; outer lobes elongated and curved dorsally alongside cerci, ending in two spines on the external face. Cerci simple, shorter than the paraprocts.

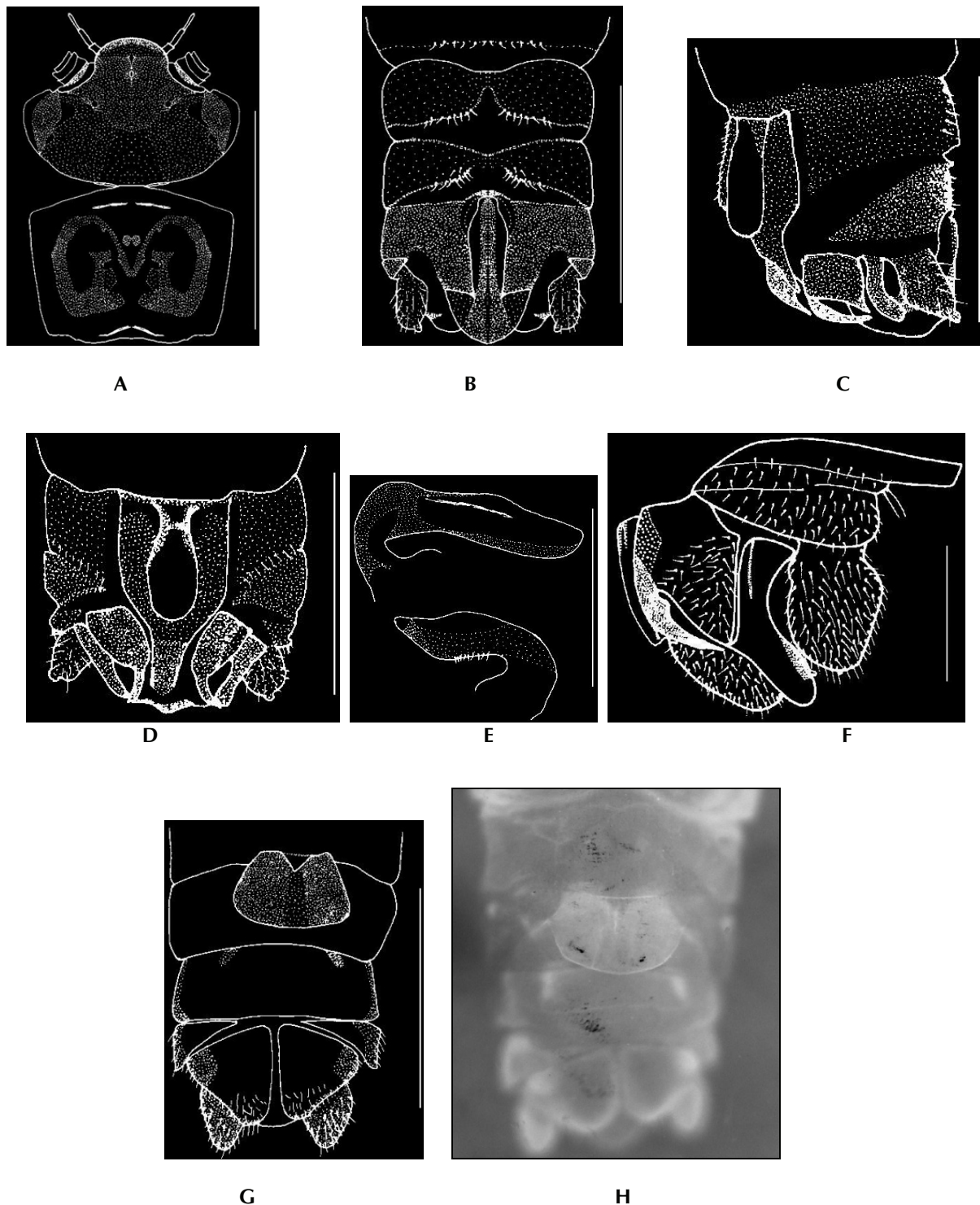
**Female diagnosis** (Figs. 3G, H). 7<sup>th</sup> sternite with a median hardly pigmented pregenital plate. 8<sup>th</sup> sternite supporting a trapezoidal subgenital plate (1/2 of the sternite width), with slightly convex posterior edge. The subgenital plate well sclerotized, except in the middle area. The vaginal lobes are hidden under the subgenital plate.

**Larval diagnosis.** General colour brown with gloss teguments (Fig. 2C). Body length: 5.58-7 mm (male); 7.27-8.09 mm (female). Cervical gills shorter with no apical constriction. Lateral gills exposed partially in dorsal view. Setal fringe of pronotum, dense, inserted on anterolateral edge. The ratio length/width of the head equal to 0.82; the pronotum equal to 0.51 (Fig. 4A). Cerci long (35 segments), darker than the body, with short setae on the margin. 6<sup>th</sup> segment as long as wide, contrary to Algerian specimens of *P. algerica*, whose 8<sup>th</sup> or 9<sup>th</sup> segment is sub-square (Aubert, 1956), and Pyrenean specimens of *P. canigolensis* (belonging to the same group) whose 7<sup>th</sup> segment is as long as wide (Zwick & Vinçon, 1993). In the male (Fig. 4B), the form of the epiproct is similar to the adult's. The last tergite has two circular, dark and symmetrical stains. 9<sup>th</sup> tergite is furnished with two fields, with short and fine silks located near the posterior edge. In the female (Fig. 4C), 9<sup>th</sup> and 10<sup>th</sup> sternites are broader than their corresponding tergites. Under-anal lobe longer than wide, with setae to the internal edge only.

**Affinities.** This species is morphologically cognate to the *corsicana* group (particularly to *P. ruffoi*), which has a Circum-Mediterranean and Caucasian distribution, with several relict or insular species (Fochetti, 1994). Nevertheless, it lacks the apical appendix of the epiproct considered as an apomorphic character of *P. corsicana* group (Zwick, 1978; Fochetti, 1994). Epiproct and paraproct shapes clearly differ from other North African *Protonemura* species, particularly due to the lack of apical appendix in the epiproct tip (as pointed above). With its epiproct shape, *P. khroumiriensis* is very similar to *P. sicula* Consiglio, 1961 from Sicily (Consiglio, 1961), but this species can be easily distinguished by its different paraproct and vesicle shapes. The female is distinguished from other North African *Protonemura* species by its trapezoid subgenital plate, narrower than those of *P. algerica*, *P. talboti*, *P. berberica* and *P. tyrrhena*, and less convex than the one in *P. ruffoi*.

**Ecology and phenology.** *P. khroumiriensis* is a common species in the Tunisian Khroumirian valley. It has been recorded by Béjaoui (2004) at several localities between 10 and 760 m a.s.l. This species preferentially colonizes temporary streams, adapting its life cycle to the flowing period of the valleys. Although this order is known by its narrow ecological valence, the ecological profile of this species shows a clear broad ecological valence towards altitude, conductivity and the velocity current (Fig. 5). This species is the most commonly recorded, constituting 22.5% of stonefly species reported to date in the area. Its flight period lasts from late winter to late spring, but differs slightly from site to site (Fig. 6).

**Etymology.** This species was named after its type locality: the "Khémir" Forest of Khroumiry.



**Fig. 3:** *P. khroumiriensis* (adult). (A) Head and pronotum; (B) tip of male abdomen in dorsal view; (C) idem in lateral view; (D) idem in ventral view (0.5 mm); (E) epiproct (0.25 mm); (F) left paraproct in  $\frac{3}{4}$  ventral view (0.25 mm); (G) tip of female abdomen in ventral view (0.5 mm); (H) idem (photo).

**Sl. 3:** *P. khroumiriensis* (odrasel primerek). (A) Glava in pronotum; (B) vrh abdomna samca, dorzalni pogled; (C) idem, lateralni pogled; (D) idem, ventralni pogled (0,5 mm); (E) epiprokt (0,25 mm); (F) levi paraprokt,  $\frac{3}{4}$  ventralni pogled (0,25 mm); (G) vrh abdomna samice, ventralni pogled (0,5 mm); (H) idem (foto).

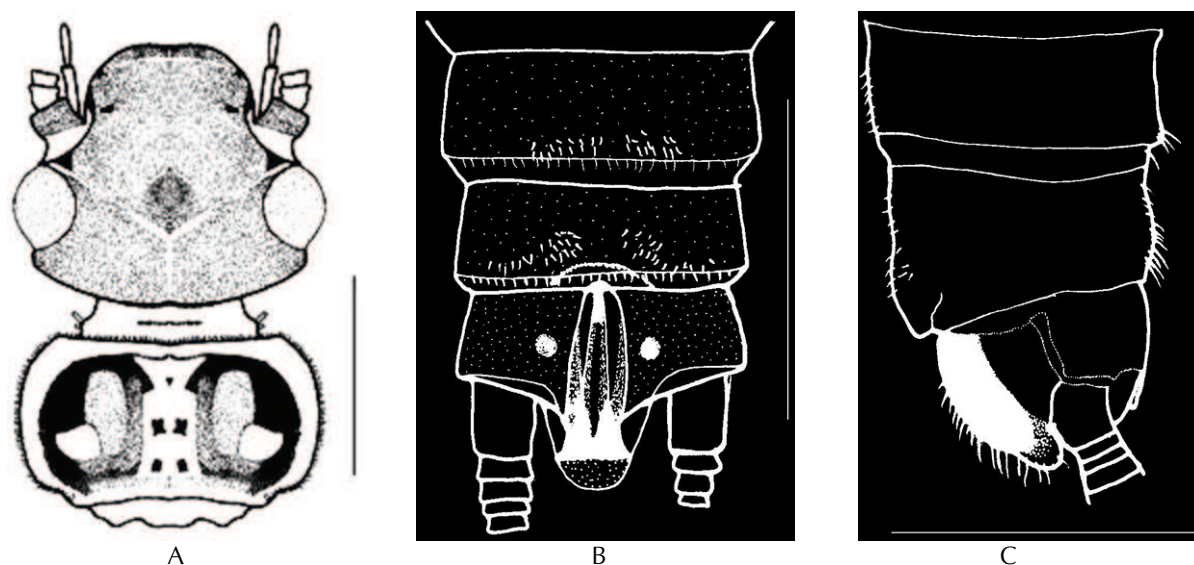


Fig. 4: *P. khroumiriensis* (larva). (A) Head and pronotum; (B) tip of male abdomen in dorsal view; (C) tip of female abdomen in ventral view (0.5mm).

Sl. 4: *P. khroumiriensis* (ličinka). (A) Glava in pronotum; (B) vrh abdomna samca, dorzalni pogled; (C) vrh abdomna samice, ventralni pogled (0,5 mm).

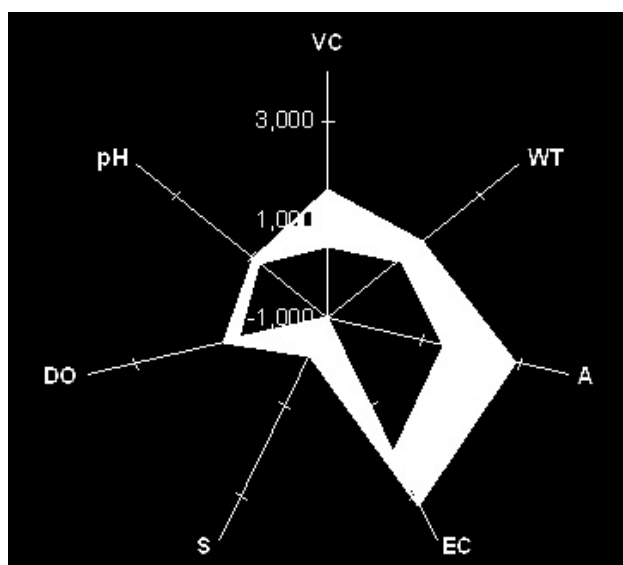


Fig. 5: Ecological profile of *P. khroumiriensis* found in Khroumirean Rivers. Legend: A = altitude (m); DO = dissolved oxygen ( $\text{mg l}^{-1}$ ); EC = conductivity ( $\mu\text{s cm}^{-1}$  at  $20\text{ }^{\circ}\text{C}$ ); S = salinity (psu); VC = velocity current ( $\text{cm s}^{-1}$ ); WT = water temperature ( $^{\circ}\text{C}$ ); pH.

Sl. 5: Ekološki profil vrste *P. khroumiriensis*, najdene v rekah gora Khroumirian. Legenda: A = nadmorska višina (m); DO = raztopljeni kisik ( $\text{mg l}^{-1}$ ); EC = prevodnost ( $\mu\text{s cm}^{-1}$  pri  $20\text{ }^{\circ}\text{C}$ ); S = slanost (psu); VC = hitrost toka ( $\text{cm s}^{-1}$ ); WT = temperatura vode ( $^{\circ}\text{C}$ ); pH.

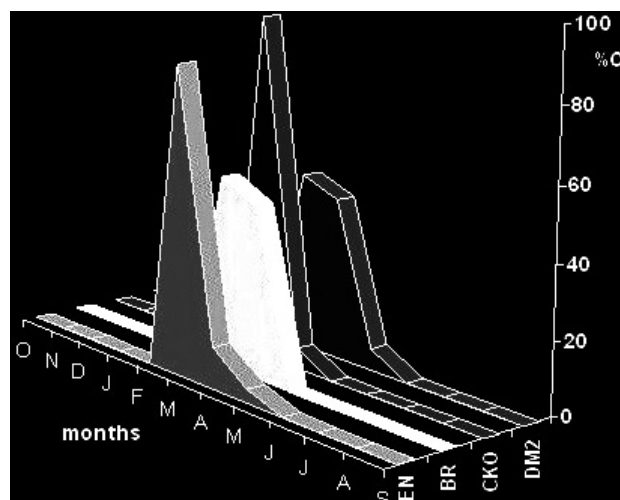


Fig. 6: Flight period of *P. khroumiriensis* from the following four streams: Ennour (EN), Bransia (BR), Châabet Kef El Ouachi (CKO) and EdDmène 2 (DM2) (%C = cumulative percentage).

Sl. 6: Obdobje letenja vrste *P. khroumiriensis* na območju štirih vodnih tokov: Ennour (EN), Bransia (BR), Châabet Kef El Ouachi (CKO) in EdDmène 2 (DM2) (%C = kumulativni odstotek).

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NOVA OPAŽANJA IN OPIS VRSTE *PROTONEMURA KHROUMIRIENSIS* SP. N. (PLECOPTERA, NEMOURIDAE) IZ TUNIZIJE

Mustapha BÉJAOUÏ & Moncef BOUMAÏZA

Unité d'Hydrobiologie Littorale et Limnique. Laboratoire de Biosurveillance de l'Environnement.

Faculté des Sciences de Bizerte. 7021 Jarzouna, Tunisia

E-mail: bejaouimustapha@yahoo.com

## POVZETEK

Pojav vrste *Protonemura khroumiriensis* sp.n. je bil zabeležen v številnih dolinah v tunizijskih gorah Khroumirian. Vrsta je morfološko sorodna vrsti *Protonemura ruffoi* iz skupine *Protonemura corsicana*, ki je razširjena okrog Sredozemlja in v Kavkazu. Posebej samec je zelo prepoznaven po obliki epiprokta brez apikalnega priveska in obliki paraproktov ter vezikla na 9. sternitu. Samico lahko prepoznamo po trapezoidni subgenitalni ploščici, ki je razen v sredinskem delu dobro sklerotizirana. Vrsta se naseljuje predvsem ob občasnih vodnih tokovih in svoj življenjski cikel prilagodi njihovim obdobjem pretoka. Obdobje letenja traja od pozne zime do pozne pomladi, vendar se malce razlikuje od območja do območja.

**Ključne besede:** *Nemouridae*, *Protonemura khroumiriensis*, dolina Khroumirian, Tunizija, nova vrsta

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