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TAXONOMIC REVISION

Adaptations to the hyporheic in Aloninae (Crustacea: Cladocera): allocation of *Alona protzi* Hartwig, 1900 and related species to *Phreatalona* gen. nov.

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Abstract Morphological study of *Alona protzi* Hartwig, 1900, *Alona phreatica* Dumont, 1983 and *Alona smirnovi* Petkovski & Flößner, 1972 reveals close affinities with *Alona labrosa* Vasiljeva & Smirnov, 1969. We separate these four species from the polyphyletic *Alona* Baird, 1843 (Anomopoda: Chydoridae). United under *Phreatalona* gen. nov., these taxa share primitive features on the limbs, together with specializations to a rheic life mode. *Phreatalona* contains some of the only true hyporheic taxa within the Cladocera. Endemism in two ancient lakes (*P. smirnovi* and *P. labrosa*) and a preference for river sediments in Europe (*P. phreatica* and *P. protzi*) suggest a long isolation from typical littoral/benthic biotopes. We discuss close links with southern vicariant *Nicsmirnovius*, the position of these (hypo) rheic chydorids within the subfamily and their affinities with *Acroperus.* We remark an independent evolution of external (habitus, postabdomen) vs. internal (limb) morphology in the *protzi*-complex. *Phreatalona* is likely tertiary in origin, evolving from a littoral alonine adapting to rheic and finally hyporheic environments. Baikal endemic *P. labrosa* is likely the most primitive species of the genus. We discuss adaptations and evolution in the hyporheic and the effect on dispersal and biogeography of *Phreatalona*.

Keywords Chydoridae · Cladocera · Hyporheic zone · Morphology · *Phreatalona* gen. n. · Stygobionts · Systematics

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Introduction

Suggestions of the polyphyletic nature of the freshwater chydorid genus *Alona* Baird, 1843 were formulated on morphological (e.g. Sinev, 2004; Van Damme & Dumont, 2008, in press) and molecular grounds (Sacherova & Hebert, 2003). *Alona* consists of several species complexes the classification of which is unstable. Necessity for global revision was stated repeatedly (e.g. Kotov & Sanuamuang, 2004). For a fuller discussion on this subject and a revision of the type species (*A. quadrangularis*), see Van Damme & Dumont (2008, in press). Taxa were attributed to different genera using limb morphology, a useful tool to unravel affinities between these externally similar micro-crustaceans (e.g. Dumont & Silva-Briano, 2000; Sinev et al., 2004, 2005; Van Damme et al., 2003). Redescriptions of marginal *Alona* species help to understand their position and delineate natural groups but a large portion still await such 'treatment'. Even within Europe, several *Alona*s are rare and poorly described. One of these is the rare *Alona protzi* Hartwig, 1900. Hartwig (1900) recognized the aberrant morphology within *Alona* and was careful about its status: "Since I am able to identify this delicate form with none of the 50 more or less well described kinds of the genus *Alona*, I describe it here as new, leaving it to the person working on this genus in the future to determine whether this is a valid species. I did not want to let the form be lost". Being the only European "*Alona*" with denticles in the posteroventral corner of its valves, *protzi* is unmistakable. It occurs in the whole of Western Europe (Smirnov, 1971; Vranovsky, 1971; Flößner, 2000), but is strongly localized with low densities, few specimens collected per site. Its ecology was poorly known (Vranovsky, 1971). Because of its rarity and low abundances, morphology was not studied and the affinities with other Aloninae are unknown. The stygophilic/stygobiotic mode of life of *A. protzi* and related species was recognized recently (Dumont, 1983; Brancelj & Dumont, 2007). A superficial similarity between *A. smirnovi, A. phreatica* and *A. protzi* was suggested by Dumont (1983) with the description of *A. phreatica*, and later by Sinev & Kotov (2000) for *A. labrosa*. Only the latter, an endemic from Lake Baikal, has to date been described in detail, including limb morphology, revealing a set of rare characters for Aloninae. Details of the other three species were unknown, though there are few drawings of limbs of *A. smirnovi* and *A. phreatica* in Alonso (1996) and Petkovski and Flößner (1972).

Since 2000, we were able to collect more specimens to check if the above-mentioned taxa are closely related as their external morphological characters suggest. Our preliminary results and distribution of *Phreatalona* were presented at the Cladocera Symposium in 2005 in Herzberg (Switzerland) (Van Damme et al., 2005). Here, we document comparative morphology of the three taxa and discuss the relationship between and the status of *A. protzi* Hartwig, 1900, *Alona phreatica* Dumont, 1983, *Alona smirnovi* Petkovski & Flößner, 1972 and *Alona labrosa* Vasiljeva & Smirnov, 1969. The trigger for this study was new material from the hyporheic realm collected within the PASCALIS project (Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface), a research project supported by the European Commission under the Fifth Framework Programme. The PASCALIS project spans six study areas, each with four neighbouring small rivers with catchment areas of approximately 100 km^2 (one each in Spain, Belgium, Italy and Slovenia and two in France). Two types of subterranean aquifers were studied: karstic systems in consolidated, fractured rocks and hyporheic zone in unconsolidated, alluvial sediments (Gibert & Deharveng, 2002; PASCALIS project, 2004). Samples taken under the PASCALIS project in porous aquifers (Brancelj & Dumont, 2007) and subsequent samplings resulted in new records for *A. phreatica* and *A. protzi* from Belgium and France. We use topotypical and new material, allocate these species to a new genus and discuss their ecology, morphology (and variability), biogeography and evolution. Study of morphological characters, especially structure and armature of thoracic limbs, enables us to establish a new genus, *Phreatalona*, presented further on in this paper.

Materials and methods

During the PASCALIS project, the Bou-Rouch method was employed to sample fauna in hyporheic zone (30–60 cm below the river bottom) and the phreatic zone (90–120 cm below the river bottom). Ten sampling locations were set for each river, evenly spaced along the river in the alluvial part. On each location, three to five sub-samples were collected (usually in a form of transect across the river's profile), and for each sub-sample a water volume of 10 l was pumped by a piston pump. On all sampling locations, nets with mesh size of 100 µm or less were used. On each sub-sampling point, first, the sample for fauna was collected from hyporheic zone, and then temperature, conductivity and oxygen concentration were measured in situ. In the next step, a steel pipe was inserted further down into the phreatic zone, where the procedure was repeated. Fauna samples from each zone were preserved in 4% formaldehyde solution before being sorted out. Final deposition of specimens was in 60% alcohol. (For more details on sampling methods and protocols see: PASCALIS project, 2004.) During an additional sampling campaign (by KVD in 2007), animals were collected using dip nets with 50 μm mesh, and fixed in 4% formaldehyde or 80% ethanol. We collected *protzi* from Heerenlaak (Belgium) and a side branch of La Lanterne (France) from a gravelly/sandy substrate by gathering the upper 20 cm of gravel by hand, rinsing it in a bucket, pouring water off through a 50 μm net and repeating this procedure. Specimens for permanent storage and dissection were transferred to a formaldehyde–glycerin mixture, mounted on glass slides, dissected under a WILD stereomicroscope at low magnification, and sealed using a rapidly solidifying varnish. Methods and materials for optical microscopic examination, SEM and annotation/numbering of the limb structures are described in Van Damme & Dumont (2007). Enumeration of setae and other limb structures is done from the epipodite towards the gnathobase, without suggestion of homology. Photo of live *P. protzi* was taken in the same way as *Anchistropus* in Van Damme & Dumont (2007), using HeliconFocus for combining photo layers.

Cladistic analysis: We used PAUP 4.0b10 (Swofford, 2000) for generating a small dendrogram illustrating morphological similarities. Branch-swapping algorithm: tree-bisection-reconnection (TBR), with random addition sequence (1000 replications). Characters, states and data matrix are given in the Supplemetary material. We performed a heuristic search and parsimony analysis with 100 bootstrap replicates and parsimony as the optimality criterion. We selected a total of 31 morphological characters for this analysis, of which 17 were limb characters. Twelve characters are considered specializations to the rheic life mode, while the function of the remaining characters is unknown. All characters were unordered and of equal weight, and starting tree was obtained via stepwise addition, default settings. We chose 11 Aloninae taxa for comparison: *Alona quadrangularis, Alona affinis, Acroperus harpae,* four species of *Nicsmirnovius* and four species of *Phreatalona*. Character choice for *Nicsmirnovius* was partly based on previous analysis by Kotov (2004). A wider sampling of *Alona* is beyond the scope of this paper, a short analysis, and our choice of characters is aimed at illustrating a rheic sub-branch of the Aloninae. Data for morphology are based on Alonso (1996), Van Damme et al. (2003), Kotov & Sanuamuang (2004), this study (*Phreatalona*) and unpublished data (*quadrangularis*). We could not perform quantitative analyses to study variability of external character (carapace and postabdomen) for the four *Phreatalona* species because of the limited number of specimens and populations of *phreatica* and *smirnovi* and none of *labrosa*.

Abbreviations

The following abbreviations, in alphabetical order, are used throughout the manuscript including Figures and Tables. A1: antennule; A2: antenna; ant: anterior; as: accessory seta; en: endite; ep: epipodite; ex: exopodite; fasc: fascicle; fc: filter comb; ft: flaming torch; gn: gnathobase; IDL: inner distal lobe; il: inner lobe; lat: lateral; nat: natatorial; ODL: outer distal lobe; P1-P6: first to sixth trunk limbs; PA: postabdomen; parth: parthenogenetic; pep: pre-epipodite; PvC: posteroventral valve corner; scr: scraper; segm: segment; ss: soft seta; tc: terminal claw. For phylogenetic analysis, CI: consistency index; HI: homoplasy index; RC: Rescaled consistency index; RI: retention index.

Definitions

We use the term “hyporheic” throughout this paper, but definitions vary according to the scientific discipline (Smith, 2005). From ecological viewpoint, the hyporheic zone is delineated by the distribution of its organisms, the hyporheos. They may contain true subterranean freshwater fauna, stygobionts and stygophiles. It is a dynamic ecotone (Sabater & Vila, 1991) intermediate between the river water above and the groundwater from saturated bedrock below, the phreatic (Valett et al., 1993; Boulton et al., 1998). The latter zone is part of the groundwater system, typically >1 m below the riverbed, where strict “subterranean” conditions prevail. There is intense exchange with both surface and underground water and even contains different zones within it (White, 1993). Differences in permeability affect movements of the hypogean fauna and their distribution. Delineation of the hyporheic and an alluvial aquifer in the floodplain, which may extend horizontally up to a few km from the main channel, is not always clear (Stanford & Ward, 1988). To hydrogeologists, the hyporheic zone is part of the groundwater system (Smith, 2005). The special habitat we are interested in for the study of *Phreatalona* is the water-saturated subsurface zone less than 1 m (typically 30–60 cm) below riverbeds of relatively small streams, where interstitial water and organisms move freely through permeable streambed deposits, between a coarse mixture of sand and gravel. This falls within the hyporheic zone.

Taxonomic account

Family CHYDORIDAE Stebbing, 1902 emend. Dumont and Silva-Briano, 1998

Subfamily Aloninae Dybowski and Grochowski, 1894 emend. Frey, 1967

Tribe Alonini Dybowski and Grochowski, 1894 emend. Kotov, 2000

Phreatalona gen. nov.

Type species. *Alona protzi* Hartwig, 1900

Etymology. The name “*Phreatalona”* is composed of “phreat-” (from *A. phreatica*) and *Alona* to indicate their adaptations to the subterranean mode of life in the *protzi*-complex.

Diagnosis

Adult parthenogenetic female. *Body* rectangular to more elongate in lateral view, with straight ventral margin; small to medium-sized animals (0.2–0.5 mm), in life translucid. *Head* protruding and rounded with short or no rostrum, three main head pores and two small pores lacking additional structures. Mandible articulation as for subfamily. *Carapace* lacking a dorsal keel, ornamentation consisting of fine wide lines or absent, no fine striation; posteroventral corner with small notch; marginal setae in posteroventral portion followed by fine spinules and up to three denticles in the posteroventral corner. *Postabdomen* 2–2.5 times as long as wide, ventral and dorsal margins relatively straight and parallel, postanal and anal portion of same dimensions, sharp preanal angle moderately to well developed; anal margin straight, postanal moderately concave, rounded dorsodistal angle; distal portion protruding, with distal notch. Marginal denticles consisting of groups of small denticles of which distalmost partly merged; lateral fascicles with spines of similar size and thickness. *Terminal claw* about as long as anal margin, straight to moderately curved, basal spine two to three times as long as width at base, implanted at some distance from claw base and reaching up to half of claw length. *First antenna* about two times as long as wide, with sensory seta implanted apically; seven apical aesthetascs subequal in length, about half as long as antennular corm and two strongly elongated aesthetascs longer than corm. *Second antenna* with spinal formula 001/(1)01 and setal formula 113/003. First exopod seta on antenna relatively long and narrow; first endopod spine reduced to fourth to third of second segment length. *Labrum* long with convex to straight margin, keel with protruding conical posterior portion and rounded tip; keel naked, lacking ventral setules. *First maxilla* with two setulated setae. Five pairs of limbs. *First limb.* First endite with two marginal setae (dorsal seta absent), second endite with three setae of which two little longer, third endite with four setae; anterior structures on endites: en1 a long seta, en2 a long seta and minute element. ODL with one slender seta, IDL with three setae of which two implanted with short or long setules in distal half. Accessory seta present and well developed. Anteriorly on corm, six to seven setule groups with more than six long setules in each group; setules in each group not decreasing in size ventrally. Ejector hooks relatively small and subequal; epipodite round, with long projection. Gnathobase a single setulated projection. *Second limb*. Exopodite with one well-developed seta; endites with eight relatively slender scrapers, first two long and slender, third shorter, last three gradually decreasing in size, all with similar denticles; gnathobasic ‘brush’ elongated triangular, gnathobase with a sensillum and three elements, filter comb with seven setae of which the first much shorter and thicker, brushlike. *Third limb*. Exopodite with quadrangular corm and seven setae in 2 + 5 arrangement; first longer than second, third exopodite seta not strongly elongated, fourth and fifth may vary in size between species (long or short), fifth and sixth setae with fine plumose setulation. External endite with three setae ($1'$–$3'$) of which first two long with fine setulation in distal half and with minute element in between, third thicker; four well-developed plump plumose setae on inner side ($1''$–$4''$); one element and four small setae on internal endite preceding gnathobase; gnathobase with a bottle-shaped sensillum, bent plumose seta with two long setae emerging from its base. Filter comb with seven long setae. *Fourth limb.* Epipodite oval-round, with projection. Exopodite small and square, with six plumose setae of which third relatively longest and fifth and sixth setae relatively narrower than others. Fourth exopodite seta strongly reduced to setulated hillock, sixth with blunt apex and subapical cluster of merged setules (not *labrosa*). Endite with marginal row of four setae, first short and scraperlike, following three strongly reduced flaming torch setae about as thick as wide and decreasing in size (not *labrosa*), and one marginal round naked sensillum implanted on the inner side of the endite; gnathobase with one long setae, bent over endite and one reduced naked element; inner side naked, filter comb with five short setae. *Fifth limb.* Epipodite round, with projection. Exopodite oval to heart shaped, mostly with deep concave margin between setae three and four; four exopodite setae, of which first two longest, same size or shorter than exopodite itself and oriented dorsally; fourth exopodite seta well developed and of similar dimensions as other three; inner lobe elongated with oval apex and long terminal setules; two thick endite setae (1′–2′) of which first little longer than second or both of same size; gnV with a process but no filter comb. *Adult male* smaller than female, with clear sexual dimorphic postabdomen with gonopores opening ventrally, subapical to terminal claw; marginal denticles consisting of unmerged groups of setules. Male IDL with three setae; thick copulatory hook with terminal rugae (ridges).

Short diagnosis of *Phreatalona* gen. n.

Small to medium-sized Aloninae with body elongated in lateral view, tapering posteriorly, three main head pores and round small pores (as opposed to *Nicsmirnovius*); head protruding rostrum short to absent, labrum strongly elongated with blunt tip, second antenna with first endopod spine reduced. Relatively short postabdomen with straight dorsal margin and straight to moderately convex ventral margin; terminal claw long, basal spine up to half its length. Five limb pairs. P1 lacking dorsal seta, IDL with three setae, anterior setae on endites one and two very long, accessory seta present, fine setule groups; P2 with well-developed exopodite seta, scrapers homogeneous in denticulation, additional element at base of scraper 1 and an elongated gnathobasic 'soft' brush, P3 with seven setae and short third exopodite seta, full set of setae in endite; P4 with reduced fourth seta (not *labrosa*). Endite lacking inner structures (autapomorphy) and with shifted receptor; P5 with four exopodite setae, elongated inner lobe and reduced gnathobase (0 setae). Males have marked postanal corner and basal spine as long as in female (Sinev & Kotov, 2000).

1. *Phreatalona protzi* (Hartwig, 1900) comb. nov.
=*Alona protzi* Hartwig, 1900: 228–230.
Type locality. Königsberg, bank of Hellsee by Biesenthai, Germany (Hartwig, 1900).
Etymology. Named in honour of Dr A. Protz, curator of Crustacea collection at Berlin in 1900 (Hartwig, 1900).

Specimens examined. Five adult parthenogenetic females, Grabensee/Salzburg, Germany, Wiener Coll., Leg. D. Flößner, 28.IV.1984, Flößner Coll., Museum Für Naturkunde, Berlin. One adult male, Hellsee by Biesenthai/Mark Brandenburg, Germany, Hartwig Collection, Leg. A. Protz, October 1889 (Hartwig, 1900), Museum Für Naturkunde, Berlin. Two adult parthenogenetic females, hyporheic Amblève River, Ile de Halleux, Walloon Region, Belgium, Leg. PASCALIS project, 30.VII.2002. One adult parthenogenetic female, hyporheic Oignin River, Charmine, Lyon, France, Leg. PASCALIS project, 18.V.2002. One adult parthenogenetic female, hyporheic Oude River, Roussillon, few kilometres from the Mediterranean coast (elevation of 13 m a.s.l.), France, Leg. PASCALIS project, 24.VII.2003. 50 adult parthenogenetic females, between moss on stones and river gravel, La Lanterne, Haute-Saône, France, 25.X.2007, Leg. K. Van Damme & D. Van Damme. 150 adult parthenogenetic females, gravel quarry Heerenlaak, adjacent to Maas River, Maaseik, Belgium, 15.VIII.2007, Leg. K. Van Damme & D. Van Damme. Two adult parthenogenetic females (in slide) from Abingdon, Berkshire, United Kingdom, 25.V.1966, Ugent Collection. Two adult parthenogenetic females (in slide) from Channel of Egridir, Turkey, Leg. H.J. Dumont, 23.VII.1973, Ugent Collection.

Redescription parthenogenetic female

Habitus (Figs. 1, 2A,B, and 3A,B)

Small to medium-sized animals, 0.32–0.43 mm, mean around 0.35 mm (n = 50, population Maaseik,

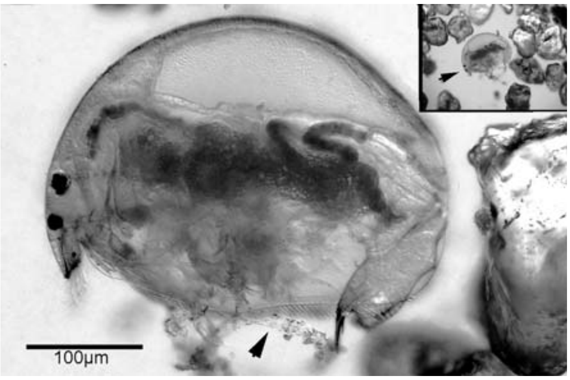


Fig. 1 Live adult parthenogenetic females of *Phreatalona protzi* (Hartwig, 1900) in sediment from hyporheic, their typical environment (from a small stream La Lanterne, Haute-Saône, France). Adult parthenogenetic female processing plant material (*arrow*). Aesthetascs are visible, projecting beyond rostrum, and typical shape of *protzi* postabdomen. Inset: same female (*arrow*), showing dimensions of sand grains vs. animal

Belgium), 0.31–0.35 mm in Vranovsky (1971), 0.32–0.42 mm in Flößner (2000). Light brown-yellow in life, colourless and transparent after fixation. Body length about 1.3–1.5 times height (Figs. 1, 2A, 3B). Dorsum strongly arched, body highest in middle, more or less ovoid, not strongly tapering posteriorly (Figs. 1 and 2A,B). Ventral margin slightly convex, with deepest point in middle (Fig. 1). Posteroventral corner with moderate notch close to posterior margin (Fig. 2I–J). In dorsal view, body compressed lacking a keel. *Head*. Ocellus and eye well developed and of similar size (Figs. 1 and 2A, E). Head shield with blunt and relatively narrow posterior margin (Fig. 2C). Rostrum present, blunt, truncated and short (Fig. 2C), aesthetascs projecting beyond its tip (Figs. 1, 2E, and 3D). In lateral view, rostrum not reaching beyond ventral carapace margin (Fig. 2E). Three main head pores (Fig. 2E) of same size, narrowly connected, PP distance about one IP distance; small pores more than half distance between midline and lateral margin of head pores (Fig. 2E).

Carapace

No ornamentation or faint wide striation; no fine striation (Figs. 2A and 3B). Number of lines 12–15. Marginal setae all similar, group in anterior and posterior third slightly longer, median group little shorter (Figs. 1 and 2A). Marginal row of 46–55 setae decreasing in size towards posteroventral corner and followed by one to four short denticles, in majority of specimens three denticles (Figs. 2I–J, 3E). Left and right valve may have a different number of denticles (also in Vranovsky, 1971). Small setules along inner margin, not arranged in groups (Figs. 2I and 3E).

Labrum (Figs. 2H and 3A)

Large, labral keel widely rounded in anterior portion and elongate triangular tip with rounded apex. About two times as long as wide. No ventral setules or denticles on labral keel. *Antennules* (Fig. 2F). Corm about two times as long as wide, sensory seta long, implanted apically. Three rows of short setules on dorsal margin. All but two aesthetascs about half as long as antennular corm and subequal in length; two aesthetascs strongly developed, longer than the antennular corm itself and reaching far beyond rostrum (Fig. 3D). *Second antennae* (Figs. 2G and 3D). Coxal setae of moderate size. Exopod without spinules or spines on second segment. Setae: 113/003, spines: 001/(1)01. First endopod spine very small, about a third of second endopod segment. Apical spines well developed about as long as or little shorter than ultimate segments. First exopod seta slender, reaching beyond terminal exopod segment. Apical exopod spine about half as long as apical endopod spine. Terminal setae subequal in length and little longer than antennal segments + coxa. Antennal muscles well developed (Fig. 2E).

Postabdomen (Figs. 2K–O and 3C)

Relatively short, dorsal margin rather straight, length about two times as long as wide (Fig. 2K). Ventral margin shorter than anal and postanal margin. Anal, preanal and postanal margins of similar length. Anal margin straight to slightly concave. Postanal margin straight to slightly tapering distally, distal margin protruding. Distal gap deep and closed (Figs. 2K–L, M and 3C). Preanal corner moderately developed, triangular, not protruding beyond postanal margin (Fig. 2K–L). In few populations, preanal corner deeper (Fig. 2N). Marginal denticles of small spines, arranged in 9–11 postanal groups (Fig. 2K). Distal postanal groups consisting of one larger denticle with nearly merged, smaller adjacent spines; marginal denticle groups closer to the anal margin in groups of

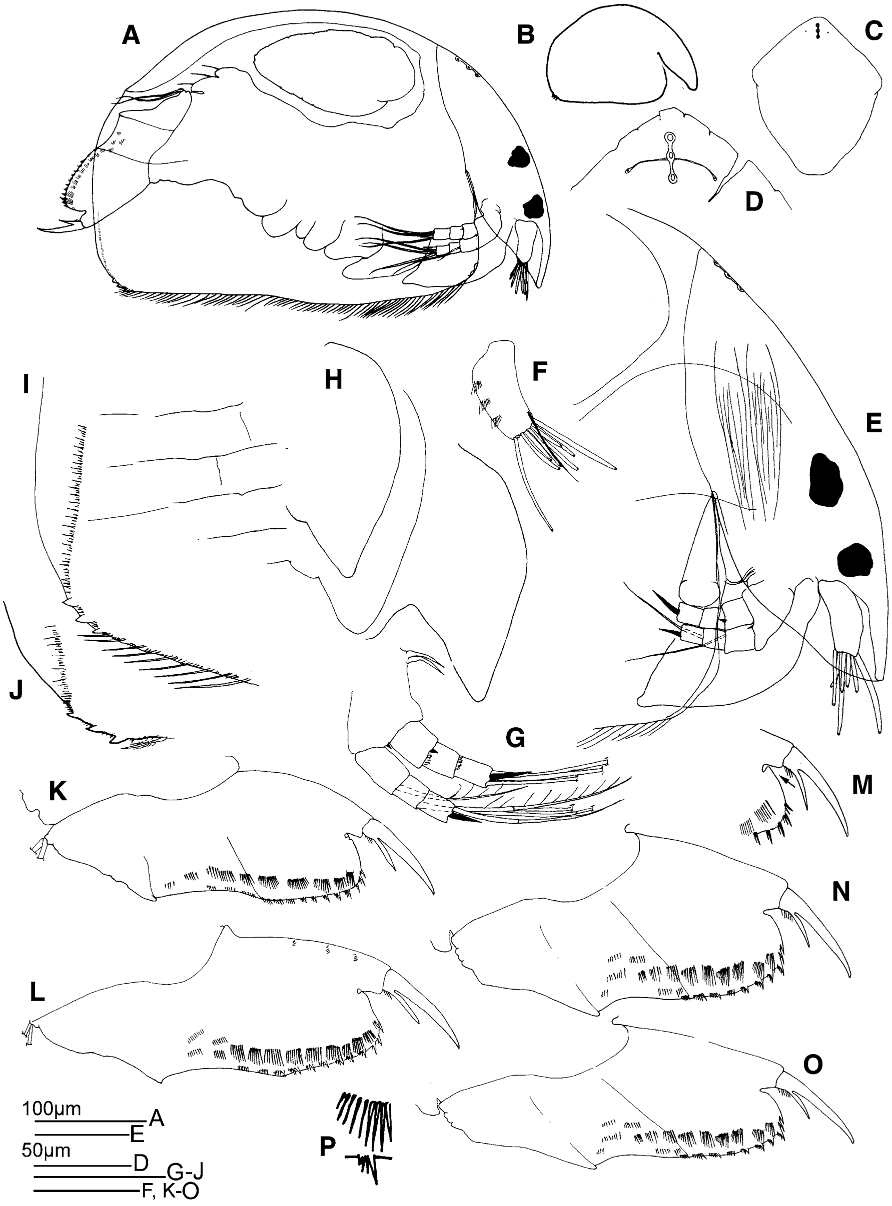


Fig. 2 *Phreatalona protzi* (Hartwig, 1900) = *Alona protzi* Hartwig, 1900. Habitus and external characters of parthenogenetic females from Belgium, Walloon Region (**A**,**B**, **D**, **F**–**I**, **K**, **M**, **P**) and Maaseik (**E**); Germany, Hellsee (**J**,**K**); UK, Berkshire (**N**) and Turkey, Egredir (**O**). **A** Habitus; **B** Body outline; **C** Headshield (after Flößner, 2000: Fig. 115c); **D** Head pores; **E** Head; **F** Antennule; **G** Antenna; **H** Labrum; **I**,**J** Posteroventral valve corner; **K**,**L** Postabdomen; **M** Idem, detail, with indication of distal embayment; **N**,**O** Postabdomen; **P** Idem, detail lateral fascicle and marginal denticle

10–13 similar, short denticles. Lateral fascicles five to eight groups postanal portion, consisting of over 15–18 parallel spinules all of similar size (no thicker distal spine) (Fig. 2P). Two to three clusters of marginal denticles and up to three rows of fascicles in anal portion (Fig. 2K,L).

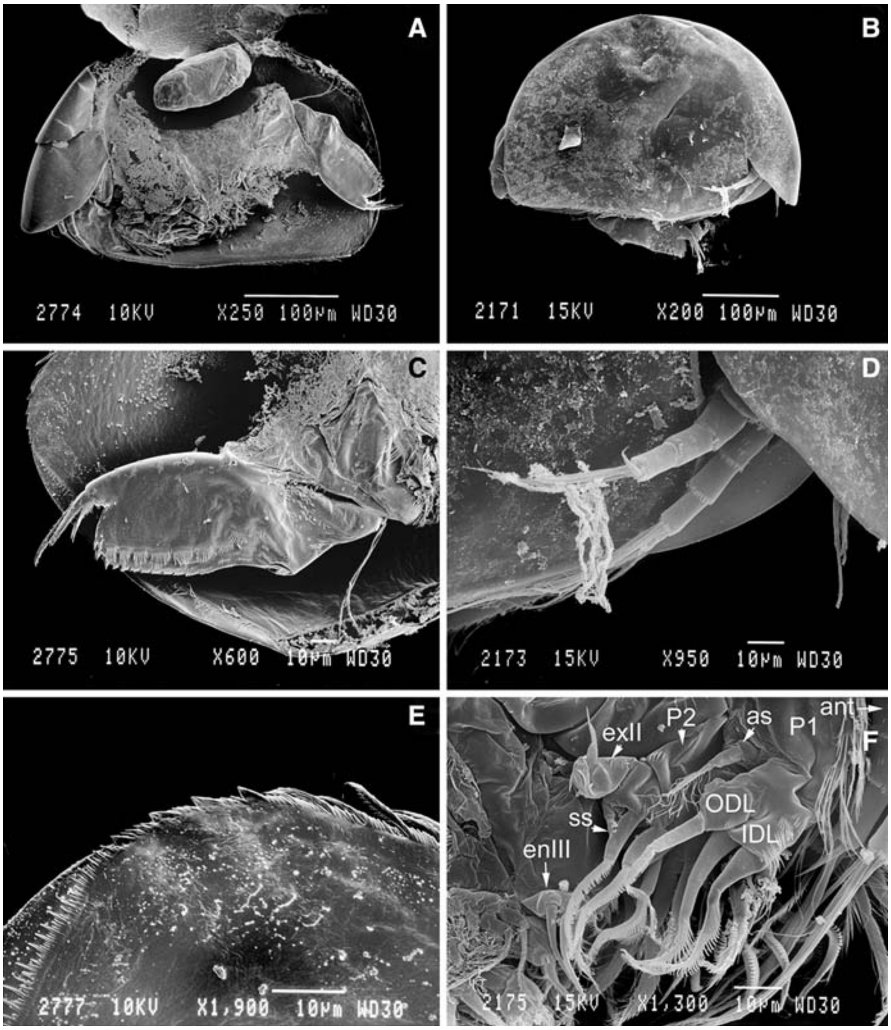
Terminal claw (Fig. 2K–M)

As long as anal margin, rather thick and evenly curved, without strong pecten. Relatively thick basal spine, slightly curved, about two times as long as claw width at base, tip reaching almost half of claw length. Group of six to eight equally long basal spinules, about one-fourth of basal spine length and continuing in setules along its dorsal margin.

Five pairs of limbs. First limb (Figs. 3F and 4A–C)

Epipodite with long projection (Fig. 4A). First endite with two slender marginal setae of similar size, second endite with three setae of which two longer

Fig. 3 *Phreatalona protzi* (Hartwig, 1900) = *Alona protzi* Hartwig, 1900. SEM of parthenogenetic females from Amblève River, Belgium. **A** Habitus, valve removed; **B** Habitus; **C** Postabdomen and inside of valve; **D** Antenna and labral keel; **E** Inner side of posteroventral valve corner with three denticles; **F** First and second limb



and subequal in size, third endite with four setae of which two posterior most longer (Fig. 3A); anterior setae on en1 and en2 long, the latter accompanied by a small element (Fig. 3B). Anterior seta on second endite more than half as long as that on first endite (Fig. 3B). ODL with one long seta as long as longest IDL seta (Fig. 3C); IDL with three setae, third seta half as long as smallest of two other setae, naked (Figs. 2F and 3C); armature of IDL setae row of equal long denticles implanted unilaterally in distal half (Figs. 2F and 3C). Accessory seta present, longer than half-length of ODL seta and finely plumose (Fig. 3C). Seven to eight anterior setule groups with more than five long slender setules in each group, all groups of similar length (Fig. 4A). Ejector hooks subequal (Fig. 4A).

Second limb (Figs. 3F and 4D,F)

Exopodite oval, with one short setulated seta (subapical), shorter than exopodite itself and implanted with short setules (Figs. 3F and 4D,E); endites with eight scrapers of which first two of similar length (Fig. 4D). At base of first seta, an additional naked soft seta is present (Figs. 3F and 4D); third scraper shorter by half of second scraper (Fig. 4F), fourth to sixth scraper of similar length as first and second (scraper five longer), seventh to eight decreasing gradually in size towards gnathobase, all relatively long and slender, increasing in thickness of proximal half towards gnathobase and all with fine denticulation (Fig. 4D); gnathobasic 'brush' strongly expanded and triangular, implanted with short setules (Fig. 4D), gnathobase with a

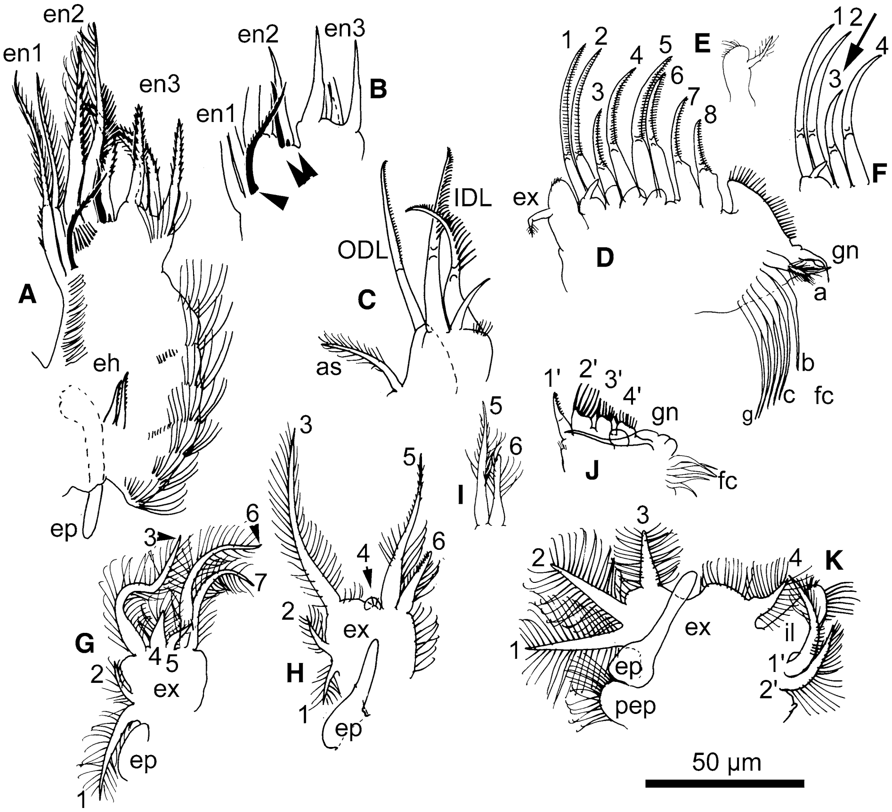


Fig. 4 *Phreatalona protzi* (Hartwig, 1900) = *Alona protzi* Hartwig, 1900. Limb characters of parthenogenetic females from Maaseik, Belgium. **A** First limb; **B** Idem, anterior side; **C** Idem, IDL and ODL; **D** Second limb; **E** Idem, exopodite; **F** Idem, morphology scrapers 1–4; **G** Third limb, exopodite; **H** Fourth limb, exopodite; **I** Idem, setae 5–6; **J** Idem, endite; **K** Fifth limb. See separate section for abbreviations, below material and methods

sensillum and three elements, of which first a bent seta; filter comb with seven long setae of which the first (a) much shorter, thicker and brushlike with setules implanted around distal half (Fig. 4D).

Third limb (Fig. 4G)

Pre-epipodite round, epipodite oval with fingerlike projection; exopodite with square corm and seven setae in 2 + 5 arrangement; first exopodite seta two times longer than second; third exopodite seta shorter than sixth exopodite seta, fourth and fifth setae short and of similar length, sixth and seventh setae narrow and long, seventh shorter than sixth. Endite as for genus.

Fourth limb (Figs. 4H–J)

Pre-epipodite round, epipodite round, with long fingerlike projection, reaching beyond centre of exopodite. Exopodite (Fig. 4H) square, with six plumose setae (2 + 4) of which first two of similar length, third seta four times as long as first, fourth strongly reduced in size to a round setuled hillock, fifth and sixth well developed. Fifth exopodite seta twice as long as sixth, the latter blunt with merged subapical setules (Fig. 4I). Endite (Fig. 4J) with marginal row of four setae, first scraperlike and shorter than first flaming torch seta. The three flaming torch setae are strongly reduced, with thick base, decreasing in size towards gnathobase, and one marginal round naked sensillum implanted on the inner side of the endite; gnathobase with one long seta, bent over endite and two reduced naked elements; on inner side, no plumose setae; filter comb with five relatively short setae (Fig. 4J).

Fifth limb (Fig. 4K)

Pre-epipodite round; epipodite round, with long fingerlike projection, reaching beyond exopodite margin. Exopodite shape broadly oval, about two times as long as wide, with strongly concave expanded margin between setae three and four; four thick exopodite setae, of which the first is longest, first two oriented dorsally, as long as exopodite length; fourth exopodite seta well developed, little

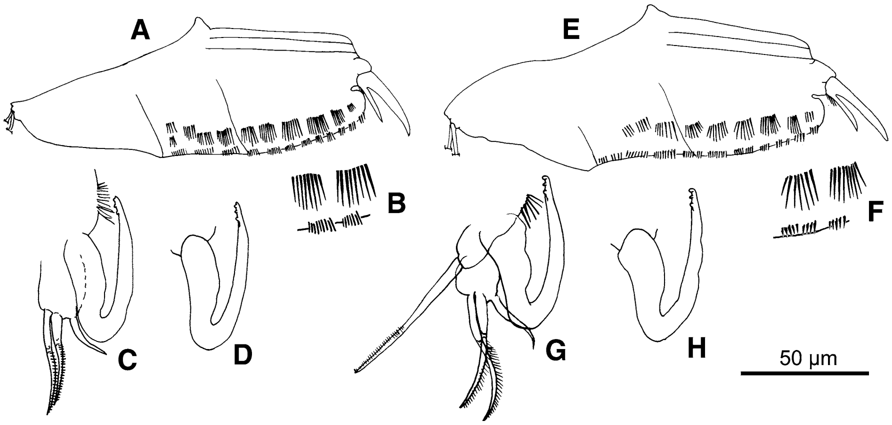


Fig. 5 *Phreatalona protzi* (Hartwig, 1900) and *Phreatalona phreatica* (Dumont, 1983), male morphology, both from type localities (Hellsee, Germany, and Auvergne, France, respectively). **A/E** Postabdomen; **B/F** Lateral fascicles and marginal denticles; **C/G** First limb, IDL and ODL; **D/H** First limb, copulatory hook

shorter than third. Inner lobe elongated oval with long terminal setules; two slender endite setae (1′–2′) of which first less than twice as long as second and bent towards inner lobe; gnathobase and filter comb absent.

Sixth limb. Absent.

Ephippial female: Unknown.

Male (Fig. 5A–D): Described in Flößner (2000), picture in Nykänen & Sarmaja-Korjonen (2007). Postabdomen and first limb based on specimen of Hartwig (1900) in collection of Flößner (2000). Postabdomen (Fig. 5A) 2.5 times as long as wide, gonopores dorsal and subapical, close to the base of the claws. Terminal claw rather thick, curved and with long basal spine reaching over half of claw; preanal corner not pronounced (Fig. 5A); lateral fascicles six postanal and four anal groups with 12–15 slender spinules each, parallel; marginal spines in groups of small unmerged spinules (Fig. 5B). First limb with IDL with three setae, hook with thick elbow and narrow tip with rugae, distal half parallel to proximal half (Fig. 5C–D).

Ecology

The most adaptive species of the genus, with relatively broad ecology. In low abundances in littoral of fast-flowing water between *Cordylophora* (Gurney, 1921), river edges; littoral in channels between *Schoenoplectus* (Vranovsky, 1971), lakes, rivers, hyporheic (Brancelj & Sket, 1990; Dumont & Negrea, 1996; Flößner, 2000), springs, quarries and on stones in small streams. The preferred habitats are saturated zones in gravel bars along rivers and hyporheic (upper 60 cm) of clear nutrient-poor streams. *P. protzi* lives in interstitial spaces of heterogeneous sediment. D.G. Frey found *protzi* in larger numbers in an underground waterconduct in the UK (Dumont, 1983). In lake littorals rich in macrophyte stands where this species is sporadically found (e.g. Nykänen & Sarmaja-Korjonen, 2007), they are probably connected with a presence of a spring, groundwater feed or inlet of small streams. During PASCALIS project, 124 specimens of *P. protzi* were collected in total (1 specimen in Belgium—hyporheic of Ourthe river; 120 specimens in Jura region near Lyon in France—hyporheic of rivers Suran and Oignin; and three specimens in Roussillon region in SW France—hyporheic of Aude river). They were all found in hyporheic zone (30–60 cm deep in gravel) with a maximum number of 30 specimens in 10 litres of pumped water. *P. protzi* appears to have a hypo-epigean life style intermediate between a stygophilic (Dumont, 1987; 1995; Dumont & Negrea, 1996) and a true stygobiont (Brancelj & Dumont, 2007) but relatively frequently washed-out into surface water, where it can extend into the littoral zone but never in high numbers. Surface waters may act as a ‘sink hole’ for this species, which can survive in places where no strong predation or unfavourable conditions appear, but is not their typical environment. Thus, it is rarely recorded via ‘classic’ sampling. It was abundantly found in two sites through aimed sampling which confirms ideas on ecology. Loc. 1: a small side-branch of La Lanterne, an oligotrophic, fast flowing stream in Eastern France (Haute-Saône), one metre deep and two metres wide. Specimens of

P. protzi were collected in large numbers (over fifty specimens in October 2007) in shaded areas between moss (*Fontinalis* sp.) on stones and from washing out gravel (Karaman-Chappuis method). The species is sympatric with the rare *Alona intermedia* (mentioned also in Hartwig, 1900), which was common between the moss and with *Unio crassus*. The latter, a bivalve, may be a good indicator of the type of streams where *P. protzi* is likely to occur (see also ecology of *P. phreatica*). Observation of live *P. protzi* from the latter locality showed that these are slow erratic swimmers. Specimens were kept alive at Ugent for several months in small 40 ml containers with *Fontinalis* moss and produced offspring without extra oxygen addition. Loc. 2: large gravel quarry (Herenlaak) near the River Maas (Maaseik, border Belgium-Germany), wherefrom water percolates into the reservoir through an aquifer. *P. protzi* was discovered here by G. Louette in the summer of 2007 and sampled subsequently. Hundreds of specimens were washed out from bare shore, from the top 10 cm of gravel or rinsing stones with epiphytes and tree roots from the reservoir's submerged 'beach', together with *Monospilus*. Within the reservoir itself, only one locality, closest to the river, contained specimens of *P. protzi*. There were no specimens in the Meuse, which shows signs of eutrophication and pollution.

Distribution

A broad range, but never before recorded in concentrations of three to four specimens per locality before this study (see also Brancelj & Dumont, 2007). Restricted to "Danubian Europe"; The Netherlands: de Molenpolder (Leentvaar, 1978), Finland, including subfossil remains, and Denmark (Silfverberg, 1999; Nykänen & Sarmaja-Korjonen, 2007), Ireland, UK (Gurney, 1921), Turkey (Güher, 2002), Lake Inkit (Behning, 1941), France (e.g. Rhone gravel bars; Dumont, 1987; Brancelj & Dumont, 2007), Germany and Poland (Flößner, 2000), Donau-delta in Rumania (Negrea, 1966), SW-Slowakia (Great Rye Island, Vranovsky, 1971), up to the Kaukasus in Georgia (Schiklejew, 1930). Not reported south of the Pyrenees (only River Ter, Sabater, 1987), Alps, Kaukasus, Taurus Mountains or east of the Ural. Record of *P. protzi* from India is a result of misidentification (Vranovsky, 1971).

Differential diagnosis

Phreatalona protzi is easily recognized by body shape and postabdomen (Figs. 1 and 2). It has the most ovoid body of the genus (body is high, dorsum arched and not strongly tapering distally, 1.3–1.5 times as long as high), antennal setae short, less than two times the length of the segments + coxa. The eye and ocellus are of similar size, well developed (diameter ocellus 0.8–1 times eye diameter and both as large as antennular width), in comparison to *phreatica* and *smirnovi,* which have a reduced eye and generally three denticles in the posteroventral corner. The latter is a good but variable (between two and four, rarely one or no denticles) and asymmetric character (Keilhack, 1911; Gurney, 1921; Vranovsky, 1971). In comparison with *phreatica* or *labrosa,*, postabdomen of *protzi* has a closed distal gap (rarely open), a convex ventral margin of postabdomen and a preanal corner that rarely exceeds the postanal postabdominal margin. There is, however, strong variation between populations of *protzi*. In comparison with *P. smirnovi*, *P. protzi* is larger, with shorter habitus (in *smirnovi* length to height is 1.6 or more), and the head has a pronounced rostrum. On limbs, *P. protzi* has modified IDL setae with long-spaced setules and a widened base, a P2 with third scraper half as long as second and a P5 with first seta as long as second.

Note

Brehm (1933) proposed the name *Alona protzi schiklejewi* Brehm, 1933, retained by Smirnov (1971), who indicated two subspecies of *P. protzi*. Behning (1941) reported *P. p. schiklejewi* from Lake Inkit, Georgia (Schiklejew, 1930; Vranovsky, 1971), stating that it differs from *P. p. protzi* in the presence of lateral fascicles on the postabdomen, six bundles with six to eight spinules each (Schiklejew, 1930; Smirnov, 1971). These bundles are definitely present in true *P. protzi* too, and this character is therefore inapplicable (Vranovsky, 1971). Published figures, however, show a short terminal claw on the postabdomen in Caucasian specimens (Schiklejew, 1930: Fig. 10). However, two specimens from Turkey, closest to *P. p. shiklejewi*, fall within the variation of typical *P. protzi* in this respect. We suggest *schiklejewi* as a synonym of *protzi* (Flößner, 2000), and not as a separate subspecies.

2. *Phreatalona phreatica* (Dumont, 1983) comb. nov.
= *Alona phreatica* Dumont, 1983
Type locality. Mountain streamlet Couze Pavin near Besse-en-Chandesse, Auvergne, France (Dumont, 1983).
Etymology. Name refers to the obligate life mode in groundwater.

Specimens examined. Eight adult parthenogenetic females, Ninglispo, Amblève, Belgium; sixteen adult parthenogenetic females and one male from hyporheic of Oignin river, Charmine, Jura region; Lyon, France, 24.7.2003; two adult parthenogenetic females, hyporheic Tech river; Correc Maureillas, Roussillon region, SW France, 17.5.2003. Three adult parthenogenetic females, hyporheic, opposite to soccer terrain, Aisne river, Bomal, Belgium, 14.8.2003. Two adult parthenogenetic females, hyporheic, Aisne, Roche-a-Frène, Bomal, Belgium; Four adult parthenogenetic females (type series, in slides) from stream Couze Pavin, Auvergne, France (Dumont, 1983), Leg. H.J. Dumont, 21–26.VI.1982, Ugent collection.

Habitus (Figs. 6A,B and 7A,B)

Medium-sized animals, 0.37–0.46 mm ($n = 18$, populations from Charmine, France) with mean length 0.4 mm, colourless and transparent. Body length 1.47–1.5 times height (Fig. 6A,B). Dorsum arched, body highest in middle, tapering posteriorly, with low posterodorsal angle (Fig. 6B). Ventral margin straight (Fig. 6A). Posteroventral corner with moderate notch close to posterior margin (Fig. 6H). In dorsal view, body compressed lacking a keel. *Head.* Ocellus and eye reduced, small and of similar size (Fig. 6A). Head shield with wavy and relatively narrow posterior margin (Fig. 6D). Rostrum wide and round without marked tip (Fig. 6C), aesthetascs projecting beyond frontal margin (Fig. 6A). Three main head pores of same size, narrowly connected, PP distance about one IP distance; small pores at about one IP distance from margin (Fig. 6D).

Carapace

Ornamentation is weak, with wide striation most pronounced in posteroventral quarter, no fine striation (Figs. 6A and 7B). Number of lines 13–15. Marginal setae with longer anterior group, median group little shorter (Fig. 6A). In total, marginal setae 50–56, decreasing in size towards posteroventral corner. On inner side of valve in posteroventral corner, setae continue in row of small setules which may be merged into denticles (Fig. 7E). These denticles do not reach beyond the valve (Fig. 7E).

Labrum (Fig. 6E–G)

Large, labral keel slightly convex in anterior portion and elongate triangular tip with rounded apex. Small notch before apex, labral keel about two times as long as wide. No ventral setules or denticles on labral keel. *Antennules* (Fig. 6I). Corm about two times as long as wide, sensory seta implanted apically and about half the size of longest aesthetasc. Two to three rows of short setules on dorsal margin. All but two aesthetascs about half as long as antennular corm and subequal in length; two aesthetascs strongly developed, longer than the antennular corm itself and reaching far beyond rostrum. *Second antennae* (Figs. 6J and 7D). Coxal setae of moderate size. Exopod without spinules or spines on second segment. Setae: 113/003, spines: 001/(1)01. First endopod spine very small, less than a third of second endopod segment. Apical spines well developed about as long as or little shorter than ultimate segments. First exopod seta slender, reaching beyond terminal exopod segment. Apical exopod spine longer than half of apical endopod spine. Terminal setae subequal in length and about twice as long as antennal segments + coxa.

Postabdomen (Figs. 6K,L and 7C)

Relatively short, dorsal margin rather straight, length about 2.5 times as long as wide. Ventral margin shorter than anal and postanal margin. Anal, preanal and postanal margins of similar length, anal margin may be little shorter. Anal margin straight to slightly concave. Postanal margin straight, distal margin protruding. Distal gap not closed. Preanal corner well developed, triangular, protruding beyond postanal margin. Marginal denticles of small spines, arranged in 9–11 postanal groups. Distal postanal groups consisting of one larger denticle with parallel adjacent spines; marginal denticle groups closer to the anal margin in groups of 6–10 similar, fine

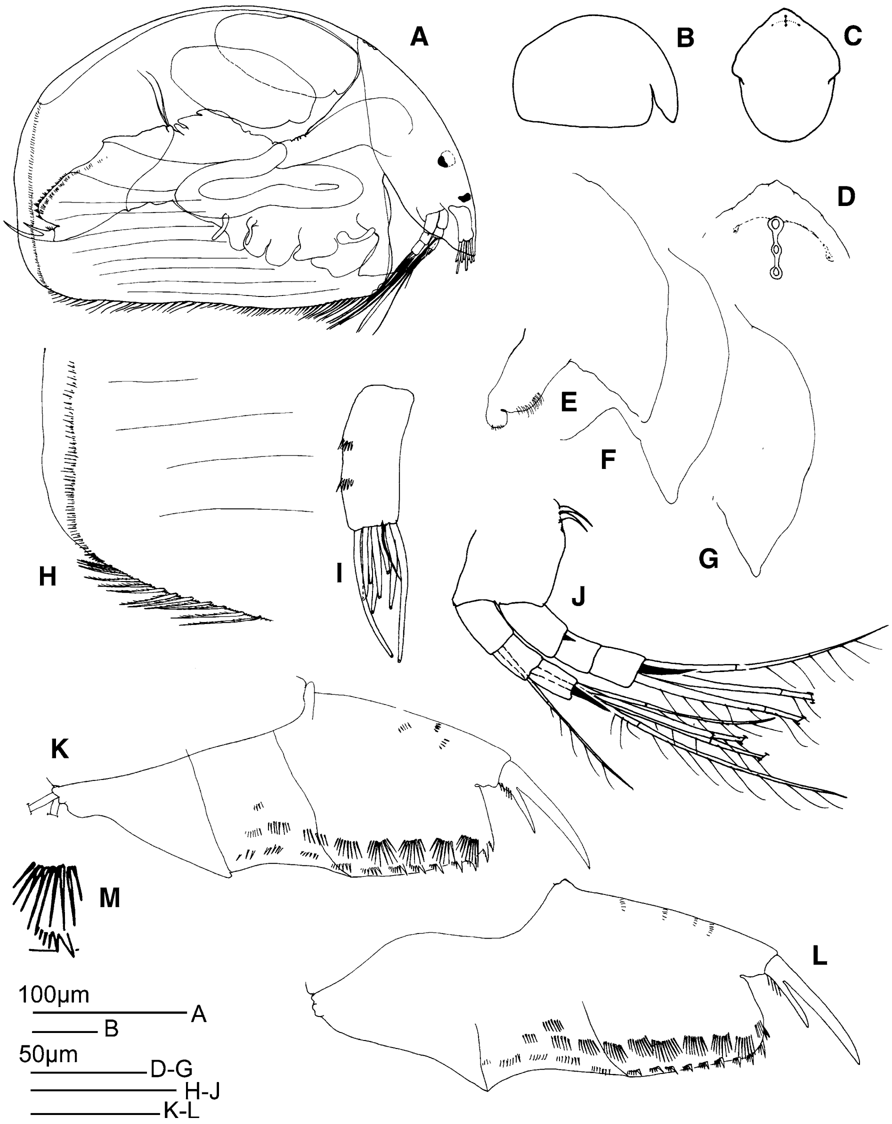


Fig. 6 *Phreatalona phreatica* (Dumont, 1983) = *Alona phreatica* Dumont, 1983. External morphology of adult parthenogenetic females from Ninglispo, France. **A** Habitus; **B** Body shape; **C** Head shield (after Alonso, 1996); **D** Head pores; **E–G** Labrum; **H** Posteroventral valve corner; **I** Antennule; **J** Second antenna; **K**,**L** Postabdomen; **M** Lateral fascicle and marginal denticle

spinules. Lateral fascicles five to seven groups in postanal portion, consisting of over 11–15 spinules all of similar size (no thicker distal spine) but not parallel (Figs. 6M and 7C). Two to three clusters of marginal denticles and up to three rows of fascicles in anal portion.

Terminal claw (Fig. 6K,L)

As long as anal margin, rather thick and straight, without strong pecten. Relatively slender and straight basal spine, about three times as long as claw width at base, tip not reaching half of claw length. Group of four to six long basal spinules, about one-third of basal spine length.

Five pairs of limbs. First limb (Figs. 7F and 8A–C)

Epipodite with long projection (Fig. 8A). First endite with two slender marginal setae of similar size, second endite with three setae of which two longer and subequal in size, third endite with four setae of similar size (Fig. 8A); anterior setae on en1 and en2 long, the latter accompanied by a small element (Fig. 8B). Anterior seta on second endite half as long as that on first endite (Fig. 8B). ODL with one long seta as long as

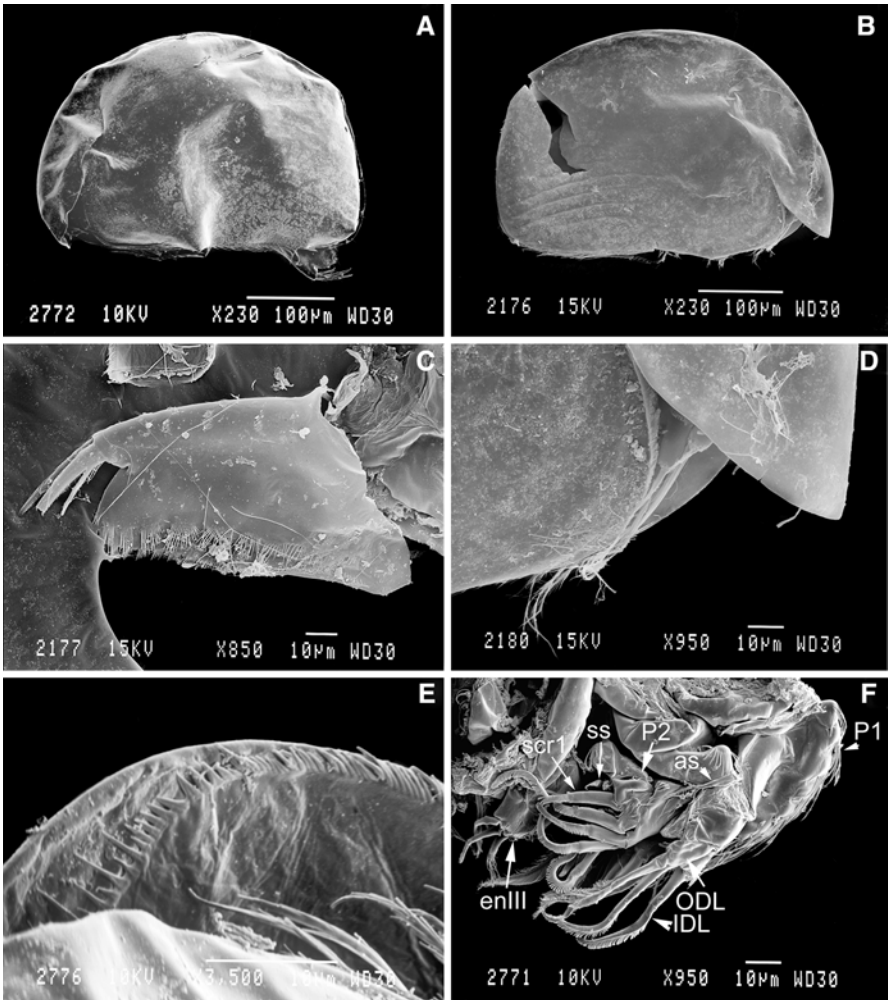


Fig. 7 *Phreatalona phreatica* (Dumont, 1983) = *Alona phreatica* Dumont, 1983. SEM of adult parthenogenetic females from Amblève, Ninglispo, Belgium, and Oignin river, Jura Region, France. **A**,**B** Habitus; **C** Postabdomen; **D** Head with antennae and labral keel; **E** Posteroventral valve corner, inner side; **F** First and second limb

longest IDL seta (Figs. 7F and 8C); IDL with three setae, third seta less than half as long as smallest of two other setae, naked (Figs. 7F and 8C); armature of IDL setae row of equal short denticles in implanted unilaterally in distal half (Fig. 8C). Accessory seta present, more than half length of ODL seta and finely plumose (Fig. 8C). Six to seven anterior setule groups with more than five long slender setules in each group, all groups of similar length (Fig. 8A). Ejector hooks subequal (Fig. 8A).

Second limb (Figs. 7F and 8D,F)

Exopodite oval, with one short setulated seta (subapical), about as long as exopodite itself and implanted with short setules (Fig. 8D,F); endites with eight scrapers of which first two of similar length (Fig. 8E). At base of first seta, an additional naked seta is present (Figs. 7F and 8E); third scraper shorter by one-fourth of second scraper, fourth to sixth scraper of similar length as first and second (scraper five longer), sixth to eight decreasing gradually in size towards gnathobase, all still relatively long and slender and of similar morphology, with fine denticulation; gnathobasic 'brush' strongly expanded, implanted with short setules, gnathobase with a sensillum and three elements, of which first a bent seta; filter comb with seven long setae of which the first (a) much shorter, thicker and brushlike with setules implanted around distal half (Fig. 8D).

Third limb (Fig. 8G–J)

Pre-epipodite round, epipodite oval with fingerlike projection; exopodite (Fig. 8G) with square corm and seven setae in 2 + 5 arrangement; first exopodite seta

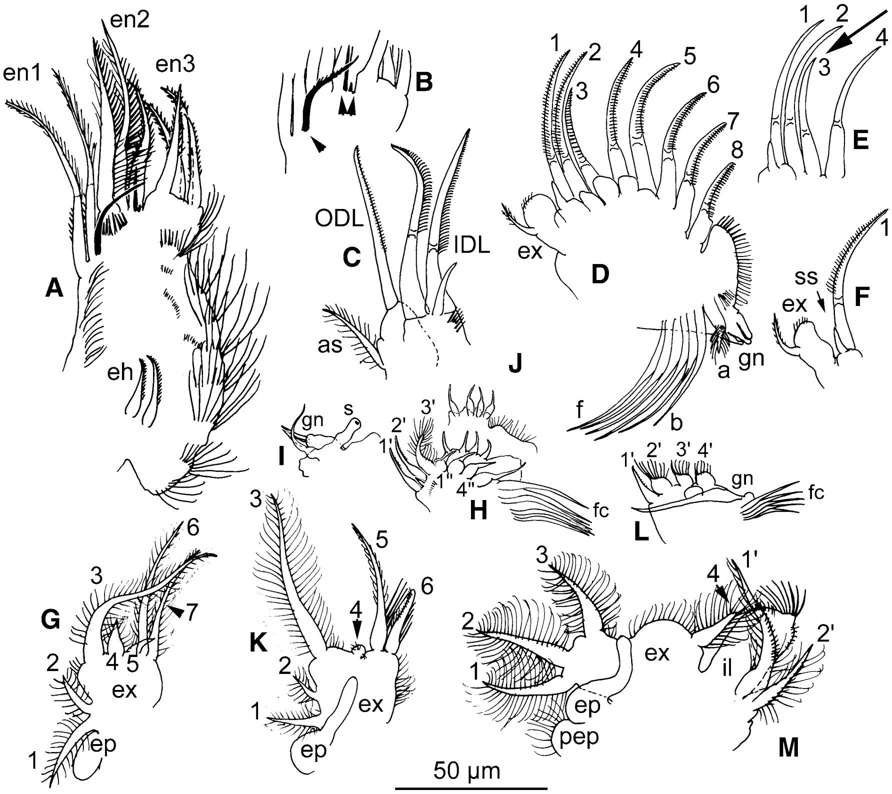


Fig. 8 *Phreatalona phreatica* (Dumont, 1983) = *Alona phreatica* Dumont, 1983. Limb morphology of adult parthenogenetic females from Amblève, Ninglispo, Belgium. **A** First limb; **B** Idem, anterior portion; **C** Idem, ODL and IDL; **D** Second limb; **E** Idem, scrapers 1–4; **F** Idem, exopodite, first scraper and adjacent soft seta (ss); **G** Third limb, exopodite; **H** Third limb, endite; **I** Idem, gnathobase; **J** Idem, outer endite setae; **K** Fourth limb, exopodite; **L** Fourth limb, endite; **M** Fifth limb

little longer than second; third exopodite seta longer than sixth exopodite seta, fourth and fifth setae short and of similar length, sixth and seventh setae narrow and long, seventh shorter than sixth (Fig. 8G). External endite (Fig. 8H) with three setae (1′–3′) of which first two slender, of similar size and with minute element in between, third (3′) short, plump and with long setules; four well-developed and thick-based plumose setae on inner side (1″–4″) of similar length; one short element and four small naked setae on internal endite preceding gnathobase (Fig. 8J); the latter with a bottle-shaped sensillum and large plumose seta with two naked setae (little shorter) at its base (Fig. 8I). Filter comb with seven long setae (Fig. 8H).

Fourth limb (Fig. 8K,L)

Pre-epipodite round, epipodite round, with long fingerlike projection, reaching beyond centre of exopodite. Exopodite (Fig. 8K) square, with six plumose setae (2 + 4) of which first two of similar size (first longer), third seta three times as long as first, fourth strongly reduced in size to a round setulated projection, fifth and sixth well developed. Fifth exopodite seta twice as long as sixth, the latter blunt with merged subapical setules. Endite (Fig. 8L) with marginal row of four setae, first scraperlike and as long as first flaming torch seta, following three ft setae strongly reduced, with thick base, decreasing in size towards gnathobase, and one marginal round naked sensillum implanted on the inner side of the endite; gnathobase with one long setae, bent over endite and two reduced naked elements; on inner side, no plumose setae; filter comb with five relatively short setae.

Fifth limb (Fig. 8M)

Pre-epipodite round; epipodite round, with long fingerlike projection, reaching beyond exopodite margin. Exopodite shape broadly oval, about two times as long as wide, with strongly concave expanded margin between setae three and four; four thick exopodite setae, of which the second is longest, first three oriented dorsally, as long as exopodite length; fourth exopodite seta well developed, little shorter than third. Inner lobe elongated oval with long terminal setules; two slender endite setae (1′–2′) of which first twice as long as second and bent towards inner lobe; gnathobase and filter comb absent.

Sixth limb. Absent

Male. Rare (Brancelj & Dumont, 2007). Described and depicted in Sabater (1987) and Alonso (1996). Postabdomen (Fig. 5E) 2.5–3 times as long as wide, gonopores dorsal and subapical, at some distance from the base of the claws. Terminal claw rather thin, straight and with long basal spine reaching over half of claw; preanal corner pronounced (Fig. 5E); lateral fascicles six postanal and three-four anal groups with 7–12 slender spinules each, widening; marginal spines in groups of small unmerged spinules (Fig. 5F). First limb with IDL with three setae, hook with thick elbow and narrow tip with rugae, distal half not parallel to proximal half (Fig. 5G,H).

Ephippial female. In Alonso (1996). Ephippium with faint yellowish tint.

Ecology

An obligate hyporheic (Dumont, 1983), found in river sediment, incapable of swimming (Dumont, 1983). In sediment of oligotrophic, clean shallow streams on sandy/gravel substrate. In Ter River (Spanish Pyrenees), *Phreatalona phreatica* reached high densities between March and September, with highest peak (ca. 800 individuals/50 l water) and a gamogenetic population in June (Sabater, 1987). During the PASCALIS project, nearly 500 specimens of *P. phreatica* were counted from the hyporheic zone at 30–60 cm below river bed. In Belgium, we counted 120 specimens from the hyporheic zone of rivers Ambleve and Ourthe, with a maximum density of 57 specimens per 10 l of pumped water. In SW France, in Rousillion region, seven specimens were collected from the hyporheic zone of river Tech. Streams in Roussillon (France) where *phreatica* was found in the hyporheic also contain the bivalve *Unio crassus*. The most numerous (incl. males and ephippial females—with pale ephippia) were in Jura region in Eastern France. More than 350 specimens were counted from hyporheic of rivers Suran, Albarine, Oignin and Valouse with a maximum density of 90 specimens per 10 l of pumped water. Only three specimens were found in the true phreatic zone (i.e. 90–120 cm below river bed). So, despite its name, it is atypical for the true phreatic and actually prefers hyporheic. In Jura region *P. protzi* and *P. phreatica* co-occurred on three locations but *P. protzi* was in lower densities compared to *P. phreatica*. It is obvious that both species can co-exist and both are tightly connected with hyporheic zone, where intensive exchange between surface and groundwater exists.

Distribution**.** Western Europe. Northern Spain (Pyrenees, Ter River), eastern France (Auvergne, Roussillon, Charmine), south of Belgium (streams Ourthe, Aisne, Amblève). Main literature: Dumont (1983), Alonso (1996), Sabater (1987), Brancelj & Dumont (2007).

Differential diagnosis. *Phreatalona phreatica* is close to *protzi*. Both may be found sympatric, but the body in *phreatica* is relatively more elongate with length 1.5 times the height and with a relatively lower dorsum (compare habitus of Fig. 1 with Fig. 6). Eye and ocellus are reduced in *phreatica*, diameter of the eye is maximally half the size of the antennular body. The antennal setae are relatively longer than in *protzi*, about 2.5 times as long as the antenna segments and coxa. In *protzi,* these setae are less than two times the length of antennal segments and coxa. *P. phreatica* lacks a protruding rostrum. This is hard to see from just the habitus, but in general the rostrum reaches less ventral than in *protzi*. *P. phreatica* rarely has denticles in the posteroventral corner of the valves and has relatively long anterior marginal setae on the valve in comparison to the other species. On the postabdomen, we listed several differences with *protzi* in Fig. 9; best characters are the open distal gap in *phreatica* (Fig. 9; number 4), a straight ventral margin, deep preanal corner reaching more ventral than the postanal margin and spread fine lateral fascicles (compare Fig. 9C with 9G). On limbs, P1 has relatively long and fine ventral setule groups implanted on the limb, and the two larger IDL setae are not modified; they have short setules and a narrow base in comparison to *protzi*. Third scraper in second limb is two-thirds as long as second scraper and exII seta is shorter than the exopodite itself.

3. *Phreatalona labrosa* (Vasiljeva & Smirnov, 1969) comb. nov.
=*Alona labrosa* Vasiljeva & Smirnov, 1969.
Synonymy and full description in Sinev and Kotov (2000).

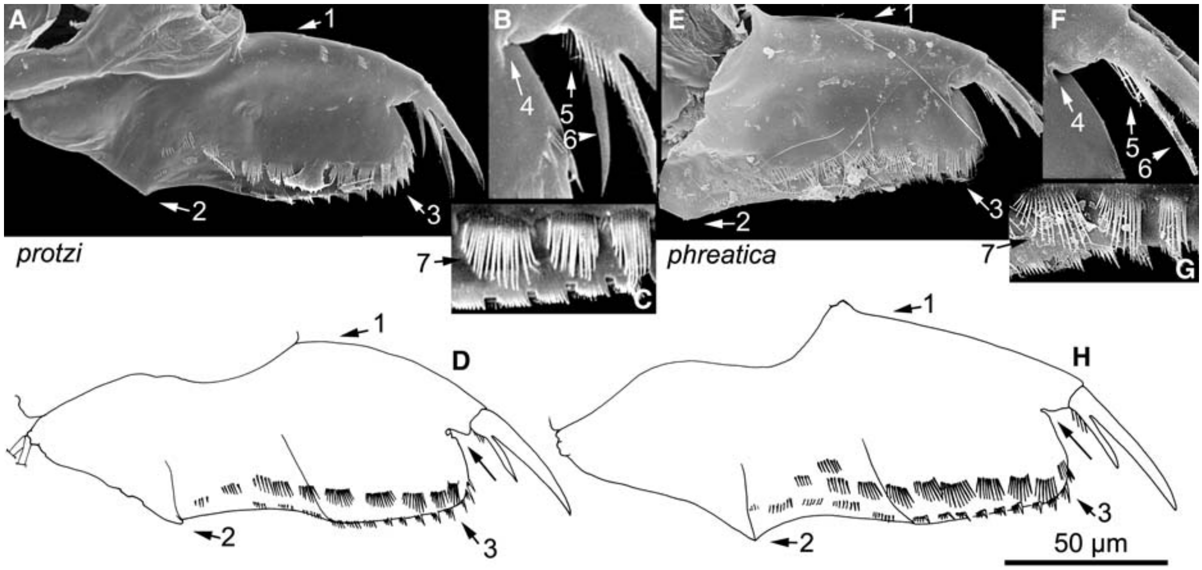


Fig. 9 Comparison of *Phreatalona protzi* (**A–D**) postabdomen with *P. phreatica* (**E–H**). *P. protzi* from Amblève, Belgium (**A–C**), and River Meuse, Maaseik, Belgium (**D**); *P. phreatica* from Ninglispo, France (**E–G**), and type locality (Besse, France) (**H**). 1. curvature ventral margin (curved in *protzi*, straight in *phreatica*); 2. depth of preanal corner (deeper in *phreatica*); 3. dorsodistal angle (more protruding in *phreatica*); 4. distal "gap" (closed in *protzi,* open in *phreatica*); 5. length and number basal spinules (more and shorter spinules in *protzi*); 6. curvature basal spine (more curved in *protzi*); 7. lateral fascicles (thicker and parallel in *protzi*, finer and divergent in *phreatica*) and marginal denticles (longer and fewer per group in *phreatica*)

Distribution and ecology. Lake Baikal, between rivers Utulic and Murina; Irkutsk Reservoir (Sinev & Kotov, 2000). Data on ecology is provided in Vasiljeva & Smirnov (1969) and Smirnov (1971). *P. labrosa* is present on sand and stones, most abundant in the open water of Lake Baikal at different depths in the littoral, between 1 and 10 m, sympatric with endemic Chydoridae *Alona setosocaudata, Kozhowia kozhowi* and *Parakozhowia baicalensis.* Highest densities in August; Vasiljeva & Smirnov (1969) collected over 400 specimens/m^2 between 6 and 10 m depth here in August 1966. There is no vegetation on the shore, the mainly stony bottom is washed by waves, and there is a vertical displacement of the littoral chydorid fauna to the open water in Baikal (Smirnov, 1971).

4. *Phreatalona smirnovi* (Petkovski and Flößner, 1972) comb. nov.
= *Alona smirnovi* Petkovski & Flößner (1972)

Type locality. In Petkovski & Flößner (1972): "Geröll des flachen litorals beim Kloster Sv. Naum am Südufer des Sees (Ohrid)" and "Loser Sand an demselben Strand, nahe der Einmündung der dort entspringenden wasserreichen Karstquellen". On submerged stones and between sand of inlet of karstic spring near Naum Monastry, southern margin of Lake Ohrid.

Etymology. Named after Dr N.N. Smirnov, diligent researcher of Cladocera systematics, Moscow (Petkovski & Flößner, 1972).

Specimens examined. Four adult parthenogenetic females, Lake Ohrid, southern margin near Monastry, Naum, Macedonia, Leg. Petkovski, IX, 1969. Coll. D. Flößner, 1970. Museum für Naturkunde, Berlin, Germany. Thirteen adult parthenogenetic females on ethanol, Lake Ohrid, Naum feeder springs (= type locality), on rocks, Macedonia, Leg. Petkovski, year unknown, Det. Petkovski, specimens provided by G. Kostoski (Director Hydrobiological Institute Ohrid), 20.VII.2007.

Habitus (Fig. 10A,B)

Small animals, 0.28–0.43 mm, colourless and transparent. In Petkovski & Flößner (1972), length 0.33–0.43 mm. Body length 1.6–1.7 times height. Dorsum arched, body highest just before middle,

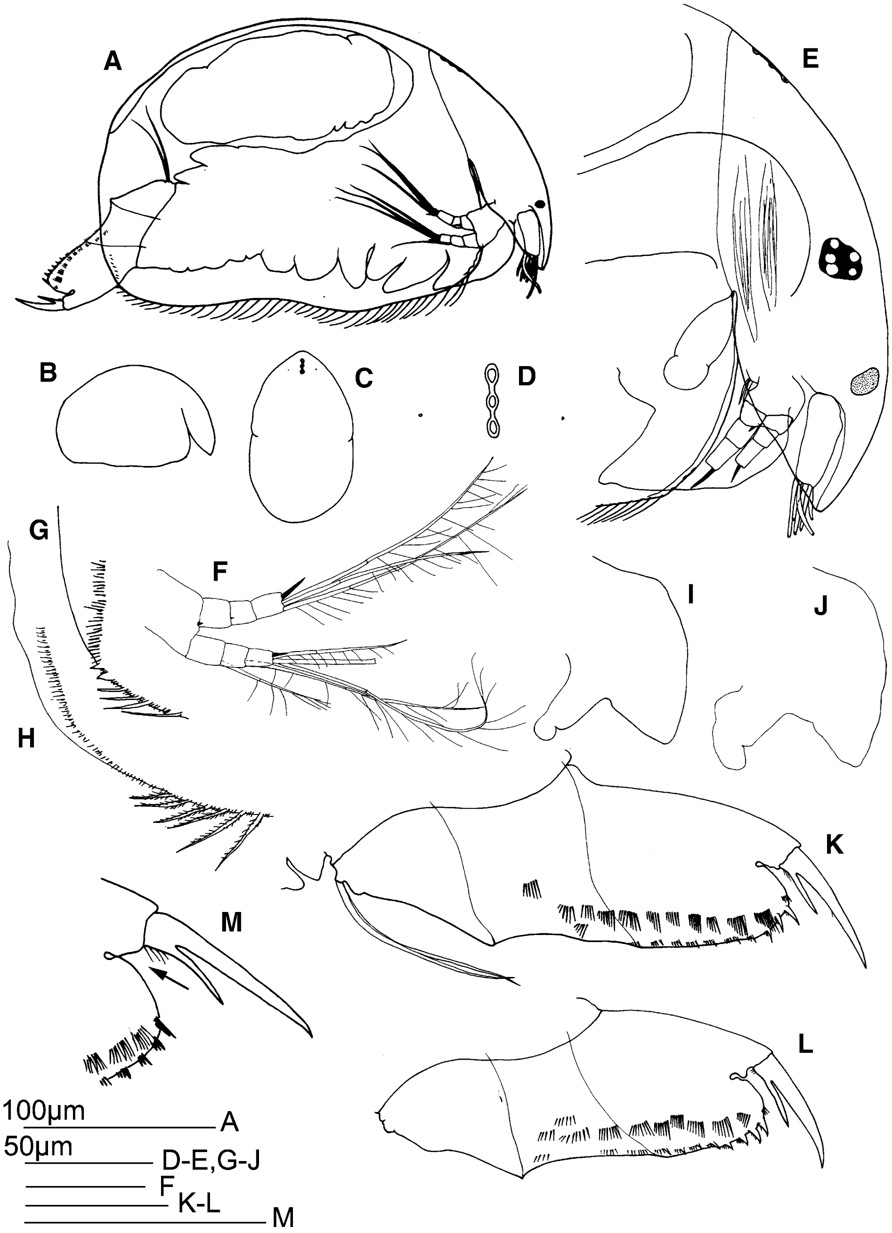


Fig. 10 *Phreatalona smirnovi* = *Alona smirnovi.* Adult parthenogenetic females, underground (from type series, underground of Naum feeder springs, Ohrid; **A**–**D**, **I**,**J**, **H**, **K**–**M**) and surface population, on rocks of Naum feeder springs, Ohrid (**E**–**G**). **A** Habitus; **B** Body outline; **C** Head shield after Petkovski & Flößner (1972); **D** Head pores; **E** Head; **F** Second antenna; **G**,**H** Posteroventral valve corner. **I**,**J**. Labrum; **K**,**L** Postabdomen; **M** Terminal claw and distal part of postabdomen

tapering posteriorly, with low posterodorsal angle (Fig. 10B). Ventral margin straight to moderately convex in anterior third (Fig. 10A). Posteroventral corner without notch close to posterior margin (Fig. 10G,H). In dorsal view, body compressed lacking a keel. *Head.* Ocellus and eye reduced, ocellus much smaller than eye (Fig. 10E). In type specimens from underground, eye is absent (Fig. 10A), but in surface material from type locality, eye is present but contains only few ommatidia (Fig. 10E). Head shield with smooth narrow posterior margin (Fig. 10C). Rostrum wide and round (not protruding) (Fig. 10C), aesthetascs projecting beyond its tip (Fig. 10E). In lateral view, rostrum reaching beyond ventral margin of carapace (Fig. 10E). Three main head pores of same size, narrowly connected and with chitinous thickening (Fig. 10D), PP distance smaller than one IP distance; small pores at about three IP distance from midline and one IP distance from margin.

Carapace

No striation (Fig. 10A). Marginal setae of similar size. In total, marginal setae 50–56, decreasing in size towards posteroventral corner (Fig. 10A). On inner side of valve in posteroventral corner (Fig. 10H), setae continue in row of small setules, which may be merged into denticles in specimens from surface (Fig. 10G). These one to two denticles may reach beyond the valve (Fig. 10G).

Labrum (Fig. 10I,J)

Large, labral keel straight to slightly convex in anterior portion and elongate triangular tip with rounded apex. No notch before apex, labral keel 1.5–2 times as long as wide. No ventral setules or denticles on labral keel. *Antennules* as for genus. *Second antennae* (Fig. 10F). Exopod without spinules or spines on second segment. Setae: 113/003, spines: 001/(1)01. First endopod spine very small, about a fourth of second endopod segment. Apical spines well developed about as long as or little shorter than ultimate segments. First exopod seta slender, reaching beyond terminal exopod segment. Apical exopod spine half of apical endopod spine. Terminal setae subequal in length and longer than antennal segments + coxa. Antennal muscles poorly developed (Fig. 10E). Antennal setae with long setules (Fig. 10F).

Postabdomen (Fig. 10K–M)

Relatively short, dorsal margin rather straight, length 2–2.5 times as long as wide. Anal margin little shorter than postanal margin. Anal margin straight to slightly concave. Postanal margin straight, distal margin strongly protruding with rounded dorsodistal angle. Distal gap deep and closed. Preanal corner short triangular, somewhat protruding beyond postanal margin. Marginal denticles of small spines, arranged in 10–11 postanal groups. Distal postanal groups consisting of one larger denticle with parallel adjacent spines, merged; marginal denticle groups closer to the anal margin in groups of 4–5 similar, fine spinules. Lateral fascicles five to seven groups in postanal portion, consisting of over 9–11 parallel spinules with slightly thicker distal spine. Two to three clusters of marginal denticles and up to three rows of fascicles in anal portion.

Terminal claw (Fig. 10M)

As long as anal margin, rather straight, without strong pecten. Relatively slender and straight basal spine, two to three times as long as claw width at base, tip just before or reaching half of claw length. Group of four to six long basal spinules, about one-third of basal spine length.

Five pairs of limbs. First limb (Fig. 11A,C)

Epipodite with long projection (Fig. 11A). First endite with two slender marginal setae of similar size, second endite with three setae of which two longer and subequal in size, third endite with four setae of similar size (Fig. 11A); anterior setae on en1 and en2 (Fig. 11B) long, the latter accompanied by a small element. ODL with one long seta little longer than longest IDL seta (Fig. 11C); IDL with three setae, third seta half as long as smallest of two other setae, naked; armature of IDL setae row of equal short denticles; accessory seta present, relatively short (less than half of ODL seta) and finely plumose (Fig. 11C). Six to seven anterior setule groups with more than five setules in each group, all groups of similar length (Fig. 11A). Ejector hooks subequal and gnathobase with single short setulated seta on globular process (Fig. 11A).

Second limb (Fig. 11D–F)

Exopodite oval, with one long setulated seta (subapical), about twice as long as exopodite itself and implanted with short setules (Fig. 11D, F); endites with eight scrapers of which first fine and two of similar length. At base of first seta, an additional naked seta is present (Fig. 11F, ss); third scraper shorter by one-third of second scraper (Fig. 11D–E), fourth to sixth scraper of similar length as first and second (scraper five longer), and last two scrapers shorter, all still relatively long and slender and of similar morphology, with fine denticulation (Fig. 11D); gnathobasic 'brush' triangular elongate, implanted with short setules, gnathobase with a sensillum and three elements, of which first a bent seta; filter comb with seven long setae of which the first (a) much shorter and brushlike with setules implanted around distal half (Fig. 11D).

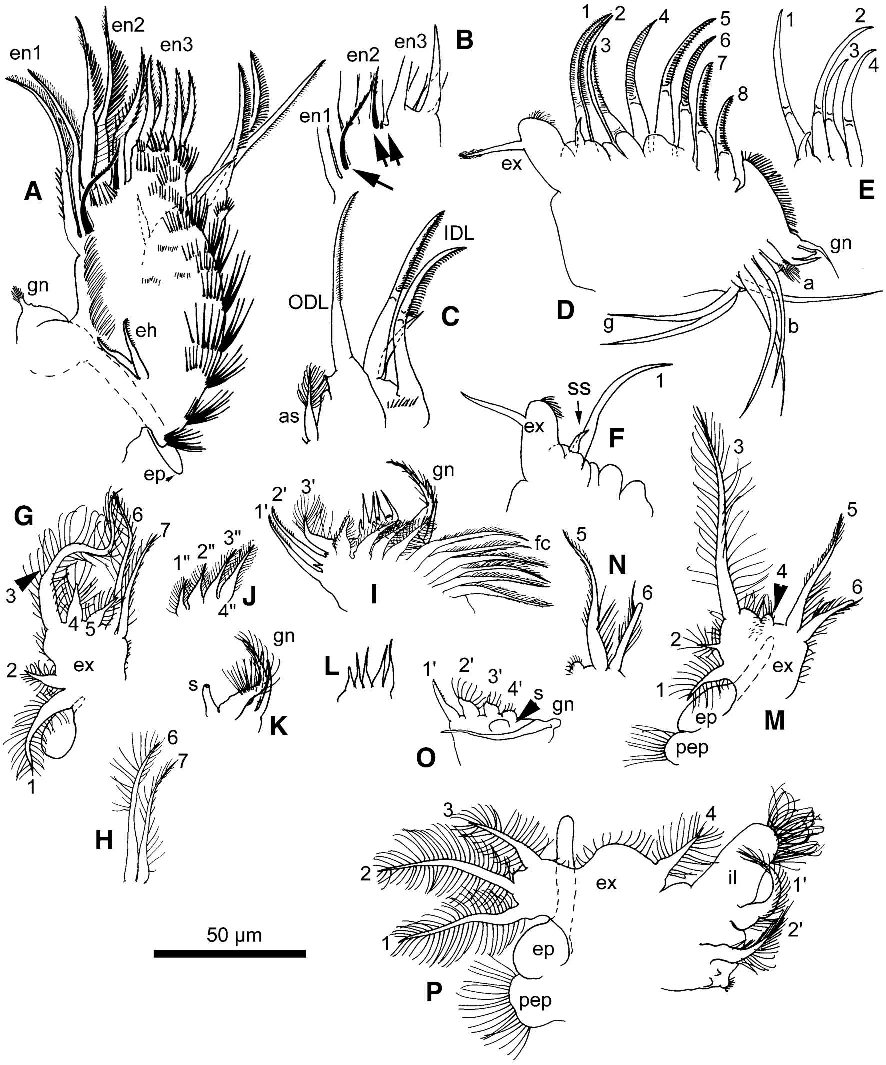


Fig. 11 *Phreatalona smirnovi* = *Alona smirnovi*. Adult parthenogenetic females from Lake Ohrid, Naum feeder springs, on rocks, Macedonia, Leg. Petkovski. Limb morphology. **A** First limb; **B** Idem, anterior portion; **C** Idem, ODL and IDL; **D** Second limb; **E** Idem, scrapers 1–4; **F** Idem, exopodite, first scraper and adjacent soft seta (ss); **G** Third limb, exopodite; **H** Idem, exopodite setae 6–7; **I** Idem, endite; **J** Idem, inner endite row; **K** Idem, gnathobase; **L** Idem, outer endite row; **M** Fourth limb, exopodite; **N** Idem, setae 5–6; **O** Idem, endite; **P** Fifth limb

Third limb (Fig. 11G–L)

Pre-epipodite round, epipodite oval with fingerlike projection; exopodite (Fig. 11G) with square corm and seven setae in 2 + 5 arrangement; first exopodite seta two times as long as second; third exopodite seta about as long as sixth exopodite seta, fourth and fifth setae short and of similar length, sixth and seventh setae narrow and long, seventh shorter than sixth (Fig. 11H). External endite (Fig. 11I) with three setae (1′–3′) of which first two slender, of similar size and with minute element in between, third (3′) short, plump and with long setules; four well-developed and stout plumose setae on inner side (1″–4″) of similar length (Fig. 11J); one element and four small naked setae on internal endite preceding gnathobase (Fig. 11I,L); the latter (Fig. 11K) with a bottle-shaped sensillum and large plumose seta with two naked setae (little shorter) at its base. Filter comb with seven long setae (Fig. 11I).

Fourth limb (Fig. 11M–O)

Pre-epipodite round, epipodite oval-round, with long fingerlike projection, reaching beyond centre of exopodite. Exopodite (Fig. 11M) square, with six plumose setae (2 + 4) of which first two of similar size, third longest, fourth strongly reduced in size to a setulated hillock, fifth and sixth well developed. Fifth exopodite seta twice as long as sixth, the latter blunt with merged subapical setules (Fig. 11N). Endite (Fig. 11O) with marginal row of four setae, first scraperlike and as long as first flaming torch seta, following three ft setae strongly reduced, with thick base, decreasing in size towards gnathobase, and one marginal round naked sensillum implanted on the inner side of the endite; gnathobase with one long setae, bent over endite and two reduced naked elements; on inner side, no plumose setae; filter comb with five relatively short setae (Fig. 11O).

Fifth limb (Fig. 11P)

Pre-epipodite round; epipodite round, with long fingerlike projection, reaching beyond exopodite margin. Exopodite shape broadly oval, about two times as long as wide, with strongly concave expanded margin between setae three and four; four exopodite setae, of which the second is longest, first three oriented dorsally and longer than length of exopodite centre; fourth exopodite seta well developed, little shorter than third. Inner lobe elongated oval with long terminal setules; two slender endite setae (1′–2′) of which first longest and bent towards inner lobe; gnathobase with a naked reduced bump and setulated hillock, filter comb absent.

Sixth limb. Absent

Distribution and ecology. Endemic to Lake Ohrid (Albania/Macedonia). Records of *A. protzi* from this lake may be *P. smirnovi*. Found in only one locality, Naum Monastry, Macedonia, in hyporheic (mesopsammom) of karstic feeder springs (Petkovski & Flößner, 1972) and on rocks at inlet of this stream, southern margin of Ohrid. The groundwater is most likely the true habitat of this species (Dumont, 1983). Its locality, Lake Ohrid is the oldest lake in Europe, formed 4–10 million years ago, supplied from surface and underwater springs (Spirkovski et al., 2001).

Differential diagnosis

Phreatalona smirnovi is a relatively small species (0.28–0.43 mm), with a mean around 0.33 mm, longest body (1.6–1.7 times as long as wide), lacking a rostrum and with eye absent or with few ommatidia, never black pigmented as in *protzi* or *labrosa*. *P. smirnovi* looks most like a small form of *phreatica* and lacks a rostrum, but its postabdomen has an arched ventral margin, the most protruding distal portion of all species and closed distal gap. This species has a chitinous thickening around the main head pores (Petkovski & Flößner, 1972). On second antenna, first endopod spine is not longer than one-fourth of the second endopod segment, in *phreatica* it reaches up to a third of this segment. The limbs (Table 1) have a short accessory seta on P1 in *smirnovi* (less than half ODL seta), exopodite seta on P2 is as long as the exopodite, third scraper is two-thirds of second scraper and on P3 the third exopodite seta is longer than the fifth.

Results of the cladistic analysis

Heuristic search yielded a single most parsimonious tree with score of best trees 51. The one retained tree was identical in topology to the tree obtained by separate bootstrap analysis (50% majrule shown here). The single tree retained from heuristic search (Fig. 12) had CI = 0.77, HI = 0.22 (excluding uninformative characters), RI = 0.84 and RC = 0.66; two of 31 characters were found parsimony-uninformative (10, 14). The bootstrap analysis produced a 50% majority rule consensus tree of 2872 trees (using tree weights) as shown in Fig. 12. The bootstrap 50% majority rule tree shows separation between true *Alona*, *A. quadrangularis* and a "rheic" branch containing *Nicsmirnovius* and *Phreatalona*. *Acroperus* is positioned basal to the latter clade. As in Kotov (2004), Neotropical *N. fitzpatricki* and Afrotropical *N. camerounensis* come out as closest relatives within *Nicsmirnovius*. For *Phreatalona, P. labrosa* is a basal taxon, followed by *protzi, phreatica* and *smirnovi*. The latter two cluster together as closest relatives, with *protzi* as their basal taxon. To check if the tree is not strongly biased by morphological specializations for the eight rheic taxa, we excluded characters that are related to their mode of life (1–3, 9–10, 16, 20–25) and reran the bootstrap analysis. This resulted in a 50% majority rule consensus tree with same topology as in the previous analysis, but with lower support for the rheic branch (54.3 instead of 65.7) and a polytomy for the four *Phreatalona* species. The latter is not shown here.

Table 1 Differences between adult parthenogenetic females of the four *Phreatalona* species, and ecology and distribution

	Phreatalona protzi-complex			*Phreatalona labrosa*
	protzi	*phreatica*	*smirnovi*	
Limb morphology				
P1 anterior setules	Short fine	Long fine	Short thick	Short thick
P1 en2 anterior seta length	>½ en1 ant seta	<½ en1 ant seta	>½ en1 ant seta	=en1 ant seta
P1 IDL setae base	Thick	Parallel	Parallel	Thick
P1 IDL setulation	Long, spaced	Short, dense	Short, dense	Short, dense
P1 naked IDL seta	½ Short IDL seta	<½ Short IDL seta	½ Short IDL seta	½ Short IDL seta
P1 accessory seta (base ODL)	Long	Long	Short	Long
P2 exopodite seta	Short (~ex)	Short (< ex)	Long (> ex)	Short (~ ex)
P2 length scrapers 1–2	Like scr. 4–6	Like scr. 4–6	Like scr. 4–6	Longer than scr. 4–6
P2 scraper 3 length	Half scraper 2	Two-thirds of scr 2	Two-thirds of scr 2	Half scr 2
P2 scraper 5 vs. 4–6	Similar length	scr 5 longer	scr 5 longer	Similar length
P3 exopodite seta 3	As long as 5	As long as 5	Longer than 5	As long as 5
P3 exopodite setae 4–5	Short	Short	Short	Long
P4 exopodite seta 4	Reduced	Reduced	Reduced	Present
P4 exopodite seta 6 apex	Blunt	Blunt	Blunt	Acute
P4 endite ft setae	Reduced	Reduced	Reduced	Developed
P5 exopodite setae 1–2 length	<Width of ex	<Width of ex	>Width of ex	>Width of ex
P5 exopodite setae 1–2 length	1 As long as 2	1 Shorter than 2	1 Shorter than 2	1 Longer than 2
P5 exopodite margin	Strongly concave	Strongly concave	Strongly concave	Straight
P5 inner (1′) seta	Long (2 × 2′)	Short	Short	Long (2 × 2′)
"External" morphology				
Body shape (lateral)	Short and high	Long, not high	Long, not high	Long, not high
Body max. length/width	1.3–1.5	~1.5	1.6–1.7	1.5–1.6
Size adult parth. female	0.32–0.42 mm	0.37–0.46 mm	0.28–0.43 mm	0.38–0.48 mm
Eye and ocellus	Well developed	Present, reduced	Eye reduced to absent	Well developed
A1 longest aesthetasc length	~A1 corm	> A1 corm	> A1 corm	~ A1 corm
A2 nat.setae/A2 segm. + coxa	Setae ~ segm.	Setae > segm.	Setae > segm.	No data
A2 apical exopod spine	~½ Endop. spine	>½ Endopod spine	½ Endopod spine	>½ Endopod spine
A2 1st exopod seta	Long	Long	Long	Short
A2 1st endopod spine	Small	Small	Minute	Minute
Rostrum	Short	Absent	Absent	Short
Carapace PvC denticles	2–3 (0)	0 (–2?)	0–2	0
PA ventral margin	Arched	Straight	Arched	Arched
PA distal margin	Protruding	Protruding	Strongly protruding	Not protruding
PA distal 'gap' (near base tc)	Closed (/open)	Open	Closed	Open
PA lat. fasc. length	Short-long	Long	Short	Short
PA lat. fasc. spread	Parallel	Spread, fine	Parallel	Parallel
Ecology	Hyporheic/littoral stygophilic/stygobiont	Hyporheic obligate stygobiont	Hyporheic/(littoral) stygobiont/(stygophilic)	Littoral not in interstitial
Distribution	'Danubian' Europe	BE, France, Spain	Lake Ohrid	Lake Baikal

Data of *Phreatalona labrosa* comb.nov. from Sinev & Kotov (2000). For abbreviations, see section below Material and methods

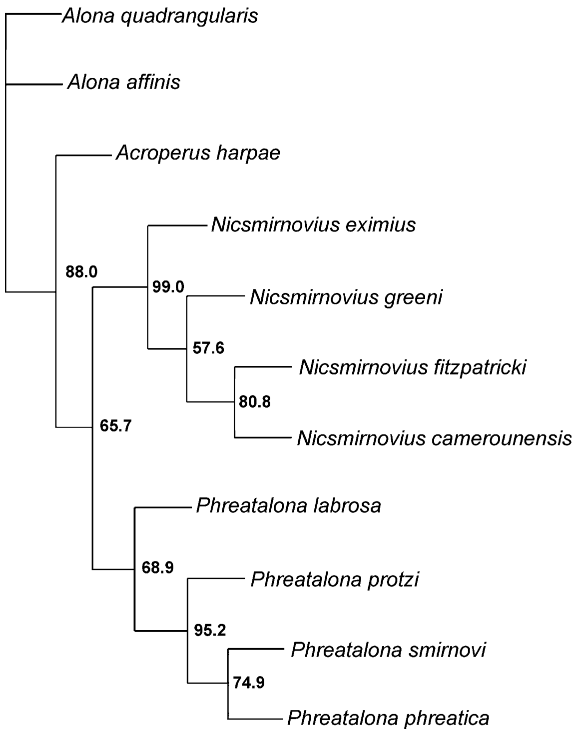


Fig. 12 Dendrogram illustrating morphological similarities of *Phreatalona* with *Nicsmirnovius* and a separation of the rheic branch from "true" *Alona* (*quadrangularis*). Within *Phreatalona, P. labrosa* is least specialized. The species may be basal to the *protzi*-group as suggested here. Single parsimonious tree, bootstrap 50% majority rule consensus of 2872 trees based on 31 morphological characters. For data and characters, see Supplementary material

Discussion

A case study of ongoing radiation: limb morphology vs. habitus and postabdomen

While *labrosa* is clearly distinct, morphological differences between *protzi*, *phreatica* and *smirnovi* are small (Table 1). Variability between *protzi* populations is considerable (e.g. postabdomen morphology), and both *phreatica* and *smirnovi* could be considered morphological extremes of the former. Adult parthenogenetic females of *phreatica* and *protzi* differ in details, but we found specimens with dubious morphology where both occur sympatrically, in Belgium and France, that could not be assigned easily to either. Also, adult males of both are almost identical. Limbs of female *protzi*, *phreatica* or *smirnovi* differ in small details, mainly on P1 and P2 (Table 1). In 'external features', however, these taxa clearly differ (e.g. body shape, rostrum, postabdomen; Table 1). We noted a variation for the shape of the postabdomen (*protzi*) and the number of denticles on the postero-ventral corner. A more 'common' morphology can be delineated, e.g. *phreatica* has no denticles and *protzi* two to four (see later). The same can be said for eye pigmentation, which may vary between taxa (*protzi/phreatica*) and populations (*smirnovi*).

Close relationships of *smirnovi–protzi–phreatica* raise the problem of assigning a taxonomic rank of these '*protzi*-forms': are they subspecies or full species? We opted for a species rank for three reasons: 1. differences in external characters indicate separation and speciation, although limb morphology and habitat preferences are close; 2. main range of the three differs (see Distribution); and 3. forms with 'dubious' morphology occur where distributions overlap (narrow belt from S Belgium into N, E and SW France—for *protzi* and *phreatica*), where a cline may exist. The latter needs more detailed study, and we aimed here to provide a detailed description of the "typical" forms for each species.

Limb investigation helps us to discriminate between conservative and more instable characters. In the *protzi*-complex, structures on P1–P2 show more variation, and therefore seem to evolve faster, than P3–P4 or the exopodite shape of P5 (Table 1). Setulation of the IDL setae, anterior setule groups on the first limb, relative length of the third scraper and exopodite seta on the second limb show more variation between these taxa than structures on the third and fourth limbs, and provide rough estimates of relatedness. Externally, the three forms differ (Table 1) in body shape and size, rostrum, length of swimming setae on second antennae and shape of the postabdomen (and lateral fascicles). The *Phreatalona protzi*-complex may be undergoing speciation, which is most obvious in external characters. In all limbs, distant position of *labrosa* is clear. In the *protzi*-complex, for example, P4 is conserved and separate from the allopatric *labrosa*, and this Baikal endemic is presumably older (see below).

The *protzi*-complex shows different speeds of evolution in limbs vs. 'external' characters. We think that evolutionary stasis in limbs is not necessarily combined with stasis in habitus and postabdomen. Evolutionary fine-tuning of external morphology and limbs may not run in parallel in these micro-crustaceans. Both may evolve independently. The disparity results from different selection pressures: habitus, antennae and postabdomen have main function in movement (crawling, swimming), whereas limbs serve mainly for food handling (selection, filter feeding, scraping). Selective pressure may not be the same for all limbs or limb characters. The exploration of new niches may be facilitated by a novelty either in limb morphology enabling another food source or in external morphology resulting in changes in mobility. Specializations as a result from different evolutionary pressures on movement vs. food handling are a key to understanding radiation and evolution in the Aloninae. This may explain why limb characters in Aloninae are sometimes so similar between taxa while habitus or postabdomen may differ strongly, or vice versa. For the *Phreatalona protzi*-complex, selection on food handling may be relatively stronger than on movement, resulting in only minor differences on limbs. Indeed, similar ecological conditions in the hyporheic zone keep limb morphology similar but allow changes in external morphology. The same habitats are frequented, and both *protzi* and *phreatica* can be found together.

Position of *Phreatalona* gen. nov. within the Aloninae

Species related to *P. protzi* have an unusual morphology for the subfamily. Sinev & Kotov (2000) list nine points of similarity between *phreatica* and *labrosa*, which can be used as features for *Phreatalona*. Superficially they may seem to have a small *Alona* habitus, but limbs differ from those of most Aloninae. Life in a rheic environment elicited adaptations of the limb structures in a completely different direction as the general littoral–benthic Chydoridae. *Phreatalona* species live mainly in the deeper part of the hyporheic, a transitional zone between surface water and phreatic zone in rivers, among coarse heterogeneous sediment. The only species not recorded from ‘subterranean’ habitats is *P. labrosa*, present in the littoral of Lake Baikal (Vasiljeva & Smirnov, 1969; Sinev & Kotov, 2000). Preliminary results of sampling gravel pits in a littoral zone on the NW part of lake Baikal (in the vicinity of limnological station Bolshoy Koty; using Karaman-Chappuis method) indicate that there are some weak subsurface inlets of seeping water, most of them quite restricted, inhabited by exclusively stygobiotic Harpacticoida and Bathynellaceae (AB, pers. observ.).

Both *protzi* and *smirnovi* also occur in surface waters, but related to a spring, gravel bed or inflow. *Phreatalona* differs from other *Alona* in general morphology and a separate evolution is clear. Main synapomorphic characters of *Phreatalona* are: 1. first antenna with elongated aesthetascs, second antenna with relatively short swimming setae and spine on first endopod segment reduced in size; 2. elongated labral keel; 3. postabdomen with a deep incision in the distal margin and protruding dorsodistal angle, relatively long basal spine, small marginal denticles arranged in clusters and lateral fascicles of similar size; 4. first limb with two well-developed anterior setae, IDL with three setae; 5. second limb with an extra seta at the base of first scraper (also in *A. hercegovinae*), all scrapers relatively slender and finely denticulated; elongated gnathobasic region; 6. third limb with seven setae of which the third is not markedly long; 7. fourth limb exopodite with reduced fourth seta and modified sixth seta with blunt apex (not *labrosa*); endite lacking a row of three inner plumose setae which are present in all Aloninae, round receptor shifted to the inner face of the endite, and a filter comb strongly reduced in size. 8. absence of a filter comb on P5 and of P6. Sinev & Kotov (2000) list characters of the antennule and postabdominal claws of males, which can be seen as additional characters for *Phreatalona*. Also, the latter authors mention an unpigmented ephippium for *P. labrosa*, unique within Aloninae. As *phreatica* also has a pale ephippium (Alonso, 1996), this unusual condition may be typical for the genus. In general, ephippia as well as males are rare in these species (Brancelj & Dumont, 2007).

Similar to many Aloninae, *Phreatalona* contains a mix of primitive and derived features on the thoracic appendages, while maintaining a general habitus. It differs in limb characters from a group of medium-sized Aloninae with small marginal denticles on the postabdomen, which lack a sixth limb and gnV (e.g. *Leberis*). For example, *Phreatalona* contains long anterior setae on the first limb and an extra soft seta basally from the first scraper on the second limb, characters considered as primitive in Aloninae. Long anterior setae on the first limb are absent in all other

Alona species with five limb pairs except for *Nicsmirnovius* and the cave inhabiting *A. hercegovinae*-group (limbs in Brancelj, 1990; Van Damme et al., 2003; Kotov & Sanuamuang, 2004). The character is however typical for a group of Aloninae with a gnathobase on P5 and a P6 (e.g. *A. affinis*-group, *A. costata*-group, *Acroperus, Graptoleberis*). Retaining these anterior setae, *Phreatalona* may be derived from the latter group but lost the sixth limb. On the other hand, specific morphological adaptations, discussed below, can be attributed to life in a (hypo)rheic environment. In general, *Phreatalona* shows remarkable reductions in limb size (exIII-V) and structure. Endite of P4 lacks three inner setae, an autapomorphy for the genus.

Adaptations to (hypo)rheic and affinities with *Nicsmirnovius*

Phreatalona shares most characters with another (hypo)rheic genus, *Nicsmirnovius* Chiambeng & Dumont, 1999. Morphology of *Nicsmirnovius* was described in detail (Van Damme et al., 2003; Kotov & Sanuamuang, 2004). Both genera have a similar mode of life and similarities seem too striking to attribute to convergence. We have listed several characters with comments in Table 2, and discuss the most important here.

Nicsmirnovius and *Phreatalona* have two long anterior setae on first limb, elongated pre-gnathobasic process on P2 (also in *Monospilus*), a short third seta on exIII (e.g. in *Monospilus, Acroperus*) and modifications of exIV setae. The function of these modified setae is unknown, but specializations are very rare in the subfamily and likely related to rheic life mode (Van Damme et al., 2003). The majority of Aloninae have two narrowed setae with pointed apex here instead of blunt setae with subapical group of clusters. Only *Alonopsis* has similar adaptations on setae of the fourth limb (Van Damme, *unpubl.*).

Total exopodite surface (exopodites, setae + setules) in relation to the body of *Nicsmirnovius* and *Phreatalona* is small compared to majority of Aloninae, especially to benthic *Alona* (*quadrangularis*-group). This is understandable from an evolutionary context: benthic Aloninae live in an oxygen-low environment and need a large 'exopodite pump', while the rheic species live in relatively oxygen-rich environments. Measurements from the hyporheic habitats sampled in Belgium and France (PASCALIS) where *protzi* and *phreatica* were found indicated that the oxygen concentration rarely drops below 50%; in the Pyrenees, *phreatica* also occurs in very clear, oxygenated interstitial water (Sabater, 1987). In this aspect, the exopodites correspond to a type of leg apparatus described by Smirnov (1971) for *Rhynchotalona* and *Monospilus*. Both these genera live on sand in open littoral, for example lake shores, where vegetation may be absent and oxygen relatively high. Both *Monospilus* and *Rhynchotalona* have passive filtering where exopodites do not make rhythmic vibrations as in the majority of Chydoridae. Limbs of *Phreatalona,* which lives in similar conditions, suggest passive filtration as well.

On enIV, *Nicsmirnovius* has a receptor shifted to the inner side like *Phreatalona*, whereas in most members of the subfamily the receptor is implanted marginally, except in four genera (*Alonopsis, Acroperus, Camptocercus* and *Graptoleberis*). Function of the shift of this sensorial structure is unknown. Both genera share a reduction on enIV of the three inner endite setae (two first strongly reduced in size in *Nicsmirnovius*, completely absent in *Phreatalona*) and a small filter comb. The combination of modifications on the fourth limb in *Phreatalona* is unique within the Chydoridae. In *Phreatalona labrosa*, the fourth limb (but even the third and fifth exopodites) is less modified than in the three taxa of the *protzi*-complex (Table 1). For example, apex of exopodite setae is sharp in *labrosa*, fourth exopodite seta not as reduced, flaming torch setae not as small and fifth exopodite is more typical for the subfamily (Table 1). In short, *P. labrosa* (Table 1) shows relatively least adaptations. This species is endemic to a lake formed 25–30 Mya ago (Baikal), where it occurs in the littoral (Fig. 13). *Phreatalona* species evolved from littoral taxa (see below), and therefore ecology of *labrosa* is relatively closer to a 'primitive' state. Morphology may reflect a primitive condition or a reversal, and we think a primitive condition is the most likely.

Phreatalona and *Nicsmirnovius* are no typical scrapers, active collectors of large particles or pure filter feeders, but are specialized in handling and processing soft, fine particulate organic matter, mycelia and decaying plant material. We observed *protzi* actively foraging and feeding on the latter (Fig. 1). Stronger reductions and modifications on

Table 2 Comparison of morphology *Phreatalona* with *Nicsmirnovius,* with comments on functionality and occurrence of these characters in subfamily Aloninae

Character	*Phreatalona*	*Nicsmirnovius*	Remarks
P1 anterior setae en1–2	+	+	Primitive condition; in *Alona, Acroperus, Camptocercus, Monospilus* etc.
P1 accessory seta (en6)	+	–	Present in most Aloninae, including *Alona*
P2 extra soft seta	+	–	Primitive; soft seta in this position in several *Alona, Acroperus,* etc.
P2 gnathobasic process	Elongate	Elongate	Unusual for Aloninae; e.g. occurs in *Monospilus* but not in *Alona*
P3 exopodite setae	Seven	Six	Seven in *Alona quadrangularis*, six in more advanced alonines (like *Alona rectangula*-group)
P3 third exopodite seta	Short	Short	Similar to penultimate exopodite seta here, much longer (about twice as long) in most Aloninae. Also short in *Acroperus, Monospilus*
P4 ex setae 5–6	5 Blunt apex	5–6 Blunt apex	Only in *Alonopsis* (setae 5–6). Sharp apex in rest of subfamily
P4 ex seta 4	Reduced, as short as wide (not *labrosa*)	Present, longer than wide	In other Aloninae never reduced
P4-P5 exopodites	Small	Small	Adaptation to rheic; in majority of Aloninae and especially *Alona quadrangularis*, exopodites larger in comparison to other limbs, where a flow is generated actively
P5 exopodite 'bilobed', with concave margin	+ (Not *labrosa*)	–	In several *Alona* (*affinis, costata, guttata*), *Acroperus* 'bilobed', not *A. quadrangularis*
P4 endite, receptor	Shifted to inner side	Shifted to inner side	Rare; only shifted in *Acroperus, Camptocercus, Graptoleberis, Alonopsis*
P4 endite, inner setae	–	Reduced in size	Always three setae here in subfamily, reduced in size in *Acroperus, Alonopsis*
P5 filter comb setae	0	0–2	No remarks
P6	–	–	Present in several *Alona*, absent in true *A. quadrangularis*
Body high in anterior portion, tapering	+ (Less in *protzi*)	+ (Least *greeni*)	Most Aloninae have oval or round body with highest point in middle. Fusiform shape may help to reduce drag in rheic environments
Head strongly protruding	+ (Less *protzi*)	+	Adaptation to interstitial/rheic; in Aloninae, head different with rostrum protruding; present in *Acroperus* and *Monospilus*
A1 one or two elongated aesthetascs	+	+	Subequal in most Aloninae; elongate in *Acroperus, Camptocercus, Alonopsis*
A1 sensory seta	Terminal	Terminal	Atypical for Aloninae, mostly implanted at 1/2d-1/3d from apex
A2 first endopod spine	Reduced	Well developed	Well developed in majority of Aloninae; longer than half segment; short in *Acroperus*
Rostrum	Short (less *labrosa, protzi*)	Short	Related to elongated aesthetascs (detection in flow). Rare in Aloninae
Labral keel	Elongate apex	Elongate (not *eximius* and *greeni*)	Rare character in Aloninae where labral keel is mostly short

Table 2 continued

Character	*Phreatalona*	*Nicsmirnovius*	Remarks
Eye reduction	+ (*phreatica, smirnovi*)	+ (*camerounensis*)	Eye reduction rare; related to underground life; in *Karualona*, *A. hercegovinae*-group, *Spinalona*, *Monospilus*
Small head pores	Simple	With 8-like structures	Simple round in majority of Aloninae, without underlying structures
PA prominent dorso-distal angle	+ (*phreatica*)	+	This causes a gap, also in *Acroperus* and *Camptocercus*; not in majority *Alona*
PA marginal postanal denticles	Very small	Very small	Large denticles, spines or serrated teeth in majority of Aloninae including *Alona*
PA lateral fascicles	Fine, similar	Fine, similar	Mostly distalmost thicker
PA male, basal spine	As long as in females	As long as in females	Rare for Aloninae and not in *Alona*

Morphology characters of *Nicsmirnovius* after Van Damme et al. (2003) and Kotov & Sanuamoang (2004), for *Phreatalona labrosa* after Sinev & Kotov (2000), and *Acroperus* and *Monospilus* after Alonso (1996). All but last characters are for parthenogenetic females

limb endites occur in a few Chydoridae specialized in feeding on soft material (e.g. animal tissue feeders *Anchistropus* and *Pseudochydorus*; Van Damme & Dumont, 2007). *Nicsmirnovius* and *Phreatalona* enter surface waters, e.g. *P. protzi*, though rarely in high numbers. We found it easy to keep *P. protzi* in culture, without the need for extra addition of oxygen. Oxygen requirements may not be the main reason for their low abundances, though these species are not adapted to benthic conditions. More likely, due to their adaptations to the interstitial, *Phreatalona* may have lost the ability to thrive in more eutrophic surface waters. This could be due to competition by other Chydoridae, predation or parasitic pressures, but temperature restraints and sensitivity to UV may also play an important role. These animals are used to relatively more stable waters of the sheltered underground, lacking conditions from surface waters, such as UV-radiation, fluctuating temperatures and subsequent warming of the body. Oscillations and temperatures are still present in the hyporheic, but are less pronounced than in surface water. We think that UV is an important factor. Indications for light sensitivity or loss of UV-protection in *Phreatalona* are: (1) clear, unpigmented/slightly pigmented ephippia in *P. phreatica* (Alonso, 1996) and *P. labrosa* (Sinev & Kotov, 2000) instead of pigmented ephippia like in majority of Aloninae; (2) inability of *P. phreatica* to survive in daylight (Dumont, 1983); and (3) abundance of *P. protzi* in littoral only in shaded localities (during our sampling campaign). The interstitium likely acted as a refuge, and today has become their true living environment.

Several *Phreatalona* species show reduced swimming abilities, observed in life for both *phreatica* (Dumont, 1983) and *protzi* (this study). It reflects in morphology of the second antenna: swimming setae in *protzi* and antennal segments in *phreatica* are short, antennal muscles are reduced in *smirnovi* and first endopod spine is reduced in all species. On the other hand, the antennal setae have long setules (depicted for *smirnovi*), which may act as sensorial equipment. *Nicsmirnovius* and *Phreatalona* share a short or no rostrum and elongated aesthetascs. An elongation of sensory equipment is an adaptation in Aloninae for tracing food in a diluted lotic environment (Van Damme et al., 2003), a common adaptation in stygobiont crustaceans.

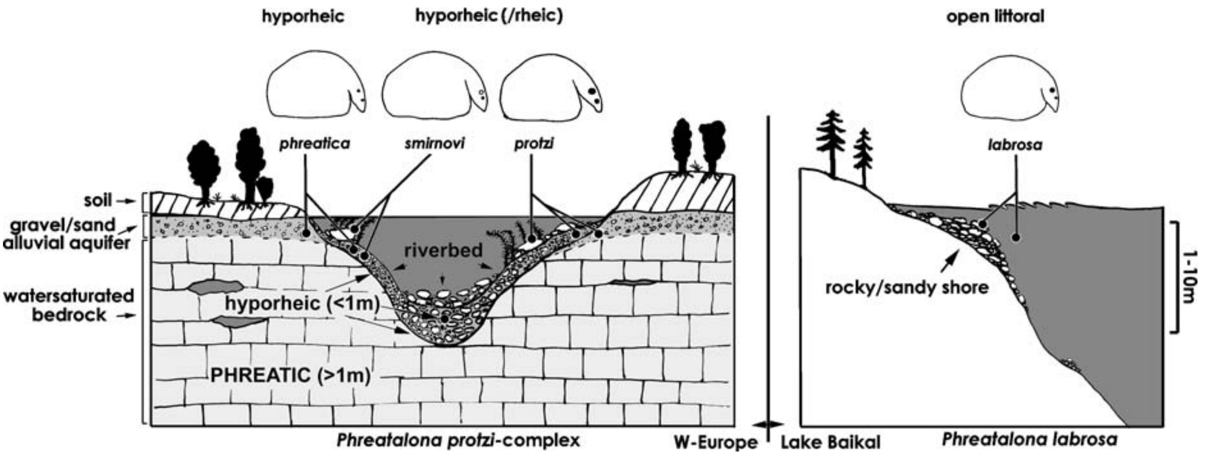


Fig. 13 Habitats occupied by different *Phreatalona* species in Western Europe and Baikal. Three species of the Western European *protzi*-complex belong to the hyporheos. Their typical habitat is the water-saturated subsurface zone of clear oligotrophic streams, in the heterogeneous sediment (gravel/sand) at 30–60 cm below the riverbed. The morphologically and geographically isolated *P. labrosa* lives in open littoral of lake Baikal. Ecology coincides with a morphological gradient: order of specialization to the hyporheic is *labrosa* < *protzi* < *smirnovi/phreatica*, from right to left in figure. *P. smirnovi* and *phreatica* have the strongest adaptations, e.g. a reduced eye and rostrum

A closer look at habitus reveals interesting adaptations. General body outline for both genera is elongate, high in anterior half and tapering posteriorly, least pronounced in *P. protzi* and *N. greeni*. In both genera, the frontal margin strongly protrudes forward and the posterior head shield margin forms a straight angle with the dorsum. This is highly unusual in Aloninae. Most species have a rectangular or oval body with highest point in the middle. The anterior head margin is not protruding forward in most Aloninae, but is inclined backwards, posterior head shield margin forming an angle of more than 90° with the dorsum. The most anterior point in most Aloninae is the rostrum, but in these rheic species it is the middle of the head. In short, heads of *Nicsmirnovius* and *Phreatalona* are built like tiny ‘battering rams’. The shape is an adaptation to life in (hypo)rheic, which exerts high mechanical forces on the frontal region. *Phreatalona* moves between heterogeneous sediment, more or less loosely distributed. There is some free space here, where particles can be displaced; otherwise animals cannot move in such environment. Only in loose sediment would these animals survive. In homogeneous, densely packed sand they cannot be found. A wide head divides forces over the frontal surface and facilitates movement through both flowing water (reduces lift) and hard substrate. The absence of dorsal keel or strong carapace ornamentation reduces the risk of damaging the carapace. Whether flow or friction, forces at this level are similar. As one of the few Chydoridae, head is strongly protruding in *Acroperus*, which, considering limb similarities, may not be a coincidence. Also *Monospilus* has a straight broad front (see Alonso, 1996). Finally, the body may be relatively fusiform in *Phreatalona* and *Nicsmirnovius* in comparison to other Aloninae. This is most pronounced in *smirnovi,* but also occurs in *labrosa*. This may be another adaptation to the rheic environment as a more fusiform shape reduces drag.

Adaptations in external morphology vary at species level (see earlier): in more typical groundwater species *P. phreatica* and *P. smirnovi*, a rostrum is absent in comparison to *P. protzi* or the littoral *P. labrosa* (Table 1). Both genera show a reduction of eye pigmentation in some species but not total blindness, a result of life in the interstitial (Dumont, 1995). The loss of eye or ocellus happened several times independently in Aloninae, e.g. in *Monospilus, Spinalona* or stygobionts like *Karualona alsafadii* and the *Alona hercegovinae*-group. In *P. labrosa* and *P. protzi*, the eye is well developed, in contrast to *P. phreatica* and *P. smirnovi*. In *Phreatalona smirnovi*, underground and surface morphologies seem to differ: specimens from groundwater (types) lacked eyes and there were no denticles on the carapace (Petkovski & Flößner, 1972), while epigean specimens from the same locality had eyes with few ommatidia and one to two denticles. Maybe, these characters are reversible. Denticles are rare in *phreatica* while in surface *protzi* they are common. Such a reduction occurs in *Karualona*, where groundwater species *K. alsafadii* lacks the strong denticles typical for the genus (Dumont, 1995). Function of the denticles is unknown in Aloninae. However, several 'costly' structures like antennae, pigmentation and carapace outgrowths may be reduced in underground habitats because of energetic limitations. Other shared characters of *Nicsmirnovius* and *Phreatalona*, which cannot be attributed to the life mode, are an elongate naked labral keel, postabdomen with long basal claw and an obtuse dorsodistal angle.

Our small dendrogram (Fig. 12) illustrates the morphological similarity between *Nicsmirnovius* and *Phreatalona* and the separation of this rheic branch from *Alona*. A larger analysis of the Aloninae may add more taxa between *Alona* and this lineage, but *Phreatalona* and *Nicsmirnovius* would likely remain near *Acroperus* in any larger morphological analysis. Inclusion of all Aloninae genera would be premature for this paper and the phylogeny of this subfamily is a complex matter. Departure of *Phreatalona* from *Alona* is however clear, and we have no doubt that this is a valid genus, separate from *Nicsmirnovius*. Existence of the rheic branch yet needs an independent test with molecular data. Robustness of this branch depends on inclusion of specialized characters in phylogenetic analysis, and there is still a possibility that these adaptations occurred twice. Our analysis with the exclusion of the specializations still showed the rheic branch but with lower support. *Nicsmirnovius* and *Phreatalona* are likely related within a wider context, retaining some primitive characters unrelated to the life mode (e.g. long anterior setae on first limb, an important character) which are reduced in many of the smaller Aloninae. The Baikal endemic *P. labrosa* is likely basal to the *protzi* group, as shown in the dendrogram (Fig. 12). *P. labrosa* is the least specialized and may therefore be the relatively more primitive species of the genus, closest to a hypothetical *Phreatalona* ancestor, which is originally a surface form (see below). *P. phreatica* and *smirnovi* seem relatively closer related, mainly due to eye reduction and the absence of a rostrum, adaptations to the hyporheic. It is still unclear whether these affinities represent actual phylogenetic relationship.

So, *Phreatalona* is far from the benthic/littoral, "true" *Alona,* and closest to *Nicsmirnovius*. Both are specialized to rheic/ hyporheic, and the affinities may result from common ancestry. Specializations of *Phreatalona* relate to life in interstitial, including eye reduction in two species, decreased swimming capacity and increase of sensorial equipment (aesthetascs). On limbs, there is a reduction of exopodite surfaces (P3–P5). Several characters, rare within the subfamily, are shared with *Acroperus* and *Alonopsis* (Table 2). Morphology suggests *Acroperus* is actually closest to *Alona* species groups with setulated labral keel and merged marginal teeth on the postabdomen. *Phreatalona* and *Nicsmirnovius* may emerge from this group, sharing an ancestor with *Acroperus*. The latter has an apparently different habitus and postabdomen, but limbs are nevertheless very close. The link even shows in ecology. *Acroperus* species enter the rheic, in littoral of oligotrophic rivers (Alonso, 1996) and sporadically into the subterranean (Brancelj & Sket, 1990). Also, *Monospilus* shares a few characters rare for the subfamily (e.g. elongated aesthetasc, long anterior setae on P1, long gnathobasic process on P2, short third seta of exIII, reduction of eye pigment). *Monospilus dispar* lives on bare sandy substrate of lake shores (Smirnov, 1971), and its adaptations suggest entering top interstitial in stagnant waters. Position of the latter, which has several adaptations to life in interstitial and primitive external features (e.g. single head pore, postabdomen with two basal spines), is unclear.

Different levels of specialization form a kind of morphological gradient within the genus (Fig. 13). Order of specialization to hyporheic, considering overall morphology, is *labrosa* < *protzi* < *smirnovi*/*phreatica*; from the littoral lacustrine *Phreatalona labrosa* over the 'stygophilic' *P. protzi* to the obligate hyporheic *P. phreatica*. In *P. smirnovi*, the comparison of two populations suggests that a switch from hyporheic to surface goes combined with phenotypical changes that may be reversible (e.g. eye pigmentation).

Age of *Phreatalona*

Our morphological analysis shows that *Phreatalona* (and *Nicsmirnovius*) separated early from the main

Aloninae trend, allowing specialization. A discussion on what is meant here by "early" may help us to estimate of the age of the Aloninae, of which no pre-Pleistocene fossils exist. Adaptations of *Phreatalona* and *Nicsmirnovius*, e.g. of limb structures, clearly derive from general alonine morphology. Both genera resemble most in limb characters to *Acroperus*, which may be related. Without doubt these chydorids originate from an ancestor with general *Alona* habitus inhabiting the freshwater littoral, exploring rheic niches and finally hyporheic. Within *Nicsmirnovius* and *Phreatalona*, surface as well as underground forms are present, with varying degrees of specialization. Adaptations to the rheic in surface waters and exploration of hard substrate in the streambed facilitated the step to hyporheic during their evolution. Several species (e.g. *Nicsmirnovius eximius*) may be found in the zone in between. *Phreatalona* shows typical features for subterraneous crustaceans, e.g. eye reduction or increased sensory equipment. Some of these adaptations may happen fast and may be reversible. As stated before, isolation of *Phreatalona* is enough for species to lose the ability in successfully recolonise surface waters, although *P. protzi* tries.

Estimated age for underground Cladocera is at least pre-Pleistocene, probably going back to the Miocene (Brancelj & Dumont, 2007). The interstitium and ancient lakes conserve groups that went extinct during glacial periods in Europe. Several stygobiont micro-crustaceans likely derive from late Tertiary surface-dwelling taxa taking shelter in the subterranean against climatic fluctuations and cooling (Brancelj & Dumont, 2007). *Phreatalona* may fit this hypothesis. Endemism in ancient lakes and the occurrence in river sediments indicate a marked isolation of *Phreatalona*.

An interesting example, *P. labrosa* is likely the most primitive member in the genus, in morphology as well as ecology. In comparison with the *protzi*-complex, the species shows least adaptations to life in the hyporheic. In Baikal, *labrosa* is found in the open water (1–10 m) and on rocks in a vegetation-free shore. Conditions are similar to those in oxygen-rich sands as with the other species, but *labrosa* is the only true surface species of a predominantly underground genus. Presence of surface forms of a hypogean group in Lake Baikal resembles a phenomenon observed in Bathynellacea (Syncarida), Mesozoic in origin. Of these micro-crustaceans found in caves and groundwater worldwide, the only two epibenthic species (*Bathynella baicalensis* and *Baicalobathynella magna*) live in Lake Baikal, at depths up to 1400 m (Kozhow, 1963). The latter species found refuge in Baikal from changing climatic conditions during ice ages, while disappearing in surface waters. The same may have happened with *P. labrosa*. Colonization of Baikal, deriving from a widespread surface form, may have happened as early as the formation of the lake (25–30 Mya) from surrounding areas where these species are now extinct (or not found yet?).

Biogeography of the *protzi*-complex (See further and Fig. 14) could be seen as another argument for pre-Pleistocene age in *Phreatalona*. Its southern boundary, determined by mountains of the Alpine Orogeny (Palaeo-Eocene, culmination in Miocene), the Pyrenees, Taurus, Caucasus and Alps, may not be coincidental. Glaciations during the Pleistocene certainly pressured colonization of groundwater (Brancelj & Dumont, 2007). Regions covered in ice sheets and several mountain chains likely acted as effective barriers, as with many groups (Hewitt, 2000, 2004). The *protzi*-complex may have never reached Spain or Italy, cut off by the Alps and Pyrenees, entering Western Europe only after these mountains were well established. Age of Lake Ohrid, home of *P. smirnovi,* could be an estimate for differentiation within the *P. protzi*-complex. As minimal estimate, it is possible that the three species of the *protzi*-complex diverged later as a result of isolations of *protzi*-like populations during glacial periods.

To conclude, we think *Phreatalona* originates from Aloninae of pre-Pleistocene origin. The morphology clearly shows a separate evolution to this environment. Most likely, its ancestors were well spread in surface waters throughout Europe, possibly even before the formation of Baikal, where the only open water/littoral species lives. The Baikal endemic also has the relatively more primitive morphology. Several populations of the *protzi*-complex likely survived adverse climate conditions in Europe finding refuge in groundwater. Brancelj & Dumont (2007) suggest the groundwater cladocerans to be relatively younger than the true karstic stygobionts (*A. hercegovinae*-group) because of fewer specializations. However, limb adaptations in *Phreatalona* are more aberrant for the subfamily than in blind *Alona*'s and

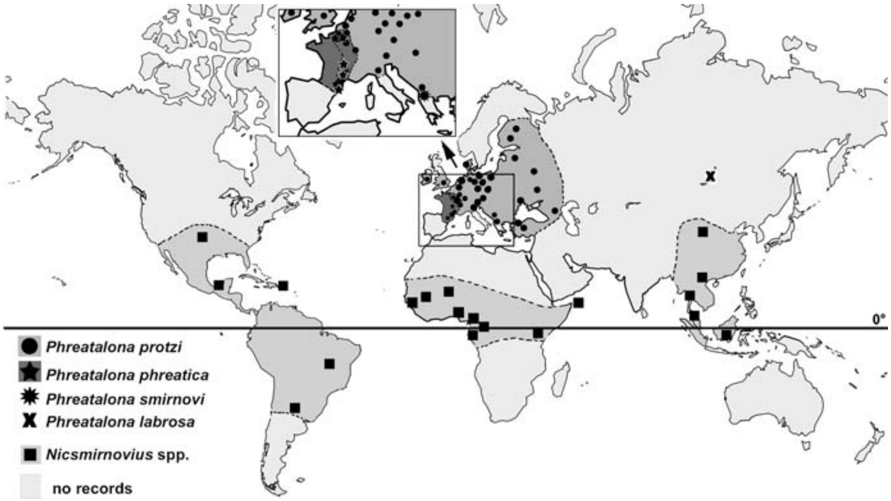


Fig. 14 World distribution of phreatic chydorids *Phreatalona* and *Nicsmirnovius;* inset: Western records of *P. protzi*-complex. Distribution of *Nicsmirnovius* after Van Damme et al. (2003), with more recent records in Neotropics (Elmoor-Loureiro, 2007) and Asia (Borneo; Dumont, *unp.*). For *Phreatalona*, after Smirnov (1971; *protzi*), Flößner (2000; *protzi*), Sinev & Kotov (2000; *labrosa*), Sabater (1987; *phreatica*), Dumont (1983; *phreatica*), Petkovski & Flößner (1972) and this study

still suggest a strong isolation. There are no morphological affinities with the true stygobiont *Alona*'s and *Phreatalona*; both entered underground separately and live in different environments. An important note for the age of Aloninae: because we lack fossils to interpret the age of different groups in this subfamily, *Phreatalona* is one of the few genera that could be used for calibration of molecular clocks. Morphological separation from their ancestor may date as far back as 30 Mya.

Distribution of *Phreatalona* and *Nicsmirnovius*

The majority of Aloninae genera and *Alona* species groups have a relatively wide cosmopolitan or southern hemisphere distribution. *Phreatalona* however is restricted to the Palaearctic. The genus is bounded to the south by the Mediterranean (Spain, Turkey) and represented here by *protzi* and *phreatica,* of which the main centre of distribution is Western Europe. The genus is so far restricted here, lacking from the Iberian Peninsula, most of Italy and Scandinavia (not S Finland). The west of France is inhabited by *P. phreatica*, the rest of Europe with *P. protzi*, but not south of the Pyrenees and Alps. Only a narrow belt of overlap of both species is known so far—along Meuse and Rhône valleys, continuing along the French Mediterranean coast to the Pyrenees. Two are endemic to ancient lakes: *P. labrosa* to Lake Baikal and *P. smirnovi* to Lake Ohrid. The former is situated mainly in old volcanic or metamorphic geology, but with some inserts of limestone, providing phreatic groundwater for springs and interstitial in a sandy littoral zone, perfect environment for *Phreatalona*. There is a "Baikalian disjunction" between this lake and 'Danubian Europe' (see below) for the genus. In the vast area in between, records are missing. It is possible that both the Ural and a large discontinuity of subsurface water in Central Asia currently act as effective barriers for these river-bound species.

Phreatalona may not disperse like surface Aloninae. The genus is adapted to permanent riverine conditions and may have lost the ability to withstand desiccation or temperature fluctuations. Furthermore, reduced pigmentation in ephippia (*labrosa, phreatica*) suggests poor adaptation to UV and no doubt affects the dispersal abilities. Their dispersal may rely predominantly on drainage basin limits and the geomorphological evolution of hydrographical networks. In this way, biogeography of the *P. protzi*-complex parallels biogeography of a large group of primary freshwater fishes restricted to rivers in 'Danubian Europe' and lacking from Peri-Mediterranea (including Spain and Italy) (Reyjol et al., 2007). Besides climatological differences, these two regions have different biogeographical history (Hewitt, 2000). Extinction, dispersal and evolution of riverine groups are strongly affected by the Last Glacial Maximum. For riverine fishes, postglacial colonization was possible from refuge populations in the Danube Basin (Reyjol et al., 2007). Biogeographical history of the *P. protzi*-complex may be similar. As discussed earlier, *Phreatalona* is likely pre-Pleistocene in origin. The current distribution pattern of the genus shows a strong influence by the Quaternary ice ages. Disjunction with Baikal suggests that *Phreatalona* was widespread in the Palaearctic and populations were likely fragmented during glaciations. The ecologically most versatile species, *P. protzi*, is widespread in Danubian Europe while all others have very limited distributions, and its expansion may be relatively recent.

Population studies of these remarkable Cladocera would be interesting, for example to investigate if separation of *protzi-phreatica* predates the Pleistocene. This would be a separate work, considering wide geographical coverage of *protzi* (e.g. UK, Scandinavia, etc.). In any case, because of the peculiar ecology and therefore a limitation to hypothetical scenarios, *Phreatalona* shows potential for the study of cladoceran biogeography and evolution.

The related *Nicsmirnovius* inhabits riverine habitats south of *Phreatalona*'s range: the Afrotropics (*N. greeni, N. camerounensis*), South East Asia, Australia (*N. eximius*) and the Neotropics (*N. fitzpatricki*) (Van Damme et al., 2003). Distributions are complementary and we can consider *Phreatalona* as a northern vicariant of the tropical *Nicsmirnovius*. As in *Phreatalona*, we think *Nicsmirnovius* largely depends on river systems for distribution. These species may however be less limited in dispersal than *Phreatalona*. *Nicsmirnovius* occurs in surface waters, though in low numbers, and ephippia are pigmented. The latter genus shows a relatively old intercontinental distribution, with separate (sub)species in tropical rainforests (e.g. *N. camerounensis*) or on isolated mountain chains of ancient islands (Socotra). We are tempted to say that the intercontinental distribution of *Nicsmirnovius* resembles that of 'Gondwana-relics'.

Gaps in distribution remain, maybe due to sampling bias. In the largest part of the Holarctic (especially North America), no hyporheic chydorids have yet been recorded. There is no reason why these should be lacking from the Nearctic and new taxa may be found here. Also, in the largest part of Inner or Eastern Asia, some remaining permanent river systems may contain surviving hyporheic chydorids. In Australia, records of hyporheic species, most likely *Nicsmirnovius*, are missing. Targeted efforts sampling interstitial riverine habitats in these regions may reveal a wider distribution or even new species. The species are however rare and may be sensitive (maybe even recently extinct in many places?) due to eutrophication/pollution.

Key to parthenogenetic females of *Phreatalona* (see also Table 1)

Note: *P. protzi* has the widest distribution and may be sympatric with *phreatica* in W Europe.

1. Eye and ocellus well developed, black, eye diameter similar to width of antennule. Rostrum short but present..................................3
2. Eye reduced, its diameter half of antennular width, to absent. If diameter similar to the antennular width, the eye has few ommatidia and is not densely pigmented but more transparent. Rostrum absent, head shield rounded................................5
3. Ocellus large, diameter 0.8–1 that of eye. Denticles in posteroventral corner of valves two to four (mostly three). Body ovoid, dorsum highly arched and mostly short, length about 1.3–1.5 times width. Postabdomen distal gap closed (see Fig. 9), IDL setae on first limb modified, with widened base and long setules, P5 with concave margin....................................*P. protzi*
4. Ocellus small, diameter 0.5–0.8 times that of eye. No denticles in PvC. Body elongate, not highly arched, length of body 1.5–1.6 times width. Postabdomen with distal gap open. IDL setae on first limb not modified (not with widened base and setules short), P5 not with concave margin. Endemic to Lake Baikal, drawings in Sinev & Kotov (2000)....................................*P. labrosa*
5. Postabdomen with distal gap open (see Fig. 9). Spinules in lateral fascicles spread, not parallel. Spine on first endopod segment of antenna small, up to one-third of second endopod segment. Body about 1.5 as long as high, not strongly fusiform............................*P. phreatica*

6. Postabdomen with closed distal gap, distal portion strongly protruding. Spinules in lateral fascicles parallel. Spine on first endopod segment of antenna up to one-fourth of second endopod segment. Body very long, its shape fusiform (tapering distally and 1.6–1.7 as long as high)................................*P. smirnovi*

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