The *Potamophylax nigricornis* group (Trichoptera, Limnephilidae): resolution of phylogenetic species by fine structure analysis

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Abstract. Applying the phylogenetic species concept and the sexual selection theory we have reviewed some natal aspects of incipient species and their accelerated evolution. How can we recognise early stages of divergence? Which selection pressures are at work during speciation? Which pathways accelerate the speed of speciation? Which kinds of trait variabilities makes difficult to find initial split criteria? Elaborating the principles of Fine Structure Analysis (FSA) and the morphological Initial Split Criteria (ISP) it was discovered that the European spring dwelling caddisfly *Potamophylax nigricornis* doesn’t belong to a single species. It represents an entire species group with seventeen peripatric species evolving on the southern peripheries of the distributional area. Four new species subgroups have been erected: *Potamophylax nigricornis* new species subgroup, *P. elegantulus* new species subgroup, *P. horgos* new species subgroup, *P. simas* new species subgroup. Eleven new species have been described: *Potamophylax apados* sp. nov., *P. fules* sp. nov., *P. fureses* sp. nov., *P. hasas* sp. novov., *P. horgos* sp. nov., *P. kethas* sp. nov., *P. lemezes* sp. nov., *P. peremes* sp. nov., *P. simas* sp. nov., *P. tuskes* sp. nov., *P. ureges* sp. nov. One *Potamophylax* sp. nov. has been differentiated and three new species status have been documented: *Potamophylax elegantulus* (Klapálek) stat. n., *P. mista* (Navás) stat. nov., *P. testaceus* (Zetterstedt) stat. nov.

Keywords. *Potamophylax nigricornis* group, Trichoptera, phylogenetic species, sexual selection, new species

INTRODUCTION

*Potamophylax nigricornis* (Pictet, 1834), this beautiful caddisfly species with faded light stripes on the dark forewing, is a widely distributed European spring dweller. It has been reported on the triangle from Lapland to Pyrenees and to Turkey. The forewing colour pattern as well as the digitate paraproct and gonopod are remarkably stable. These elongated periphallic organs have dominated the scope of the routine identification practice so much, that the high diversity of the phallic organs remained undetected (Figs. 1–3). Emerging theory of sexual selection has started to
focus on structural details of the phallic organ and on the internal structures of the female genital chamber. Colour divergences on the distributional peripheries induced early description of new species or variants: Phryganea testacea Zetterstedt, 1840 (= P. testaceus (Zetterstedt, 1840) stat. n.) from Lappland; Stenophylax nigricornis var. elegans Klapálek, 1899 (= P. elegantulus (Klapálek, 1899) stat. n.) from Bosnia; Stenophylax nigricornis var. mista Navas 1918, and Stenophylax aculeatus Navas 1919, both from Spain (Pyrenees) (P. mista (Navás, 1918) stat. n.) The second valid species Potamophylax schmidt Ma-rinkovic was recognised also by its remarkable dark striped forewing from Bosnia-Herzegovina.

The doubts about identification and misidentifications of recently collected dark striped specimens from various mountains in the Balkan Peninsula and paler specimens from France (Oláh 2010) have initiated our present study to find other traits than just pigmentation in order to separate and to distinguish among taxa. Application of the phylogenetic species concept and the sexual selection theory inspired us to initiate the examination of the fine structures of the phallic organ as well as of the vaginal sclerite complex directly involved in mating. This theoretical background accompanied with our principle of Fine Structure Analysis (FSA) revealed that Potamophylax nigricornis doesn’t belong to a single species. It represents an entire species group composed of seventeen peripatric species evolving on the southern peripheries of the distributional area. This intense population differentiation possibly developed during the Pleistocene and probably in sexual and ecological speciation processes.

Molecular genetics has created spectacular resolution in research of speciation processes. Our FSA on male and female structures directly involved in sexual selection processes of mating offers even higher resolution level per value for money (Oláh et al. 2012, Oláh & Ito, 2013). It was shocking to realise that seventeen phylogenetic species hid under a single species. However more diversity of this species group is still to be discovered because the present synopsis was based upon limited material that was put together from set aside materials. A target oriented systematic collection project will produce more new taxa. It seems that juvenile incipient taxa of the phylogenetic species concept are strongly camouflaged by the former species ranking concept in spite of the spreading practice of routine DNA sequencing.

Here we have reviewed some pointed areas of theoretical relations concerning the birth of the incipient species and their accelerated evolution. We are working in alpha taxonomy and not familiar in details about the rapid progress of molecular genetics. Therefore this review was just prepared for our own understanding in the following questions. How can we recognise early stages of divergence? Which selection pressures are at work during speciation? Which pathways accelerate the speed of speciation? Which kinds of trait variabilities make it difficult to find initial split criteria?

THEORETICAL PART

According to the phylogenetic species concept the species are “branches in the lines of descent” (Darwin 1859). Species are entire population lineages and not only a stage in the lineage divergence. Species has been transferred from the hierarchy of taxonomic rank to the hierarchy of biological organisation (deQueiroz 2011). Species is not a taxonomic rank, but it is a level of biological organisation. Placing discrete boundaries on the continuous process of diversification is a misleading practice. Species are organised and permanently changing realities during their entire life span, from initial separation to extinction. Molecular genetics proves that reproductive barriers are semipermeable to gene flow and species can differentiate despite ongoing interbreeding (Hausdorf 2011). The basic challenge however remained both for taxonomist and for geneticist how to find initial split criterion. That very point in the continuum of morphological or molecular divergence where or when a new species is born. In taxonomy we have to search stable structural entities for initial split criterion. This allows co-
vering the two roles of taxa in the phylogenetic species concept.

1) As entity of the evolution theory, manifesting the organising continuum of living world.

2) As lineage, describing biodiversity and reflecting the pattern of the organised life (Oláh et al. 2012).

**How to recognise early stages of divergence?**

To detect initial separation, the very beginning of the morphological divergence, the birth of a new divergent structure is a great, but promising challenge to alpha taxonomist. Two ways to study the genetic and structural changes in the time course of speciation have been differentiated and symbolized by spyglass and magnifying glass (Via 2009).

1) Retrospective analysis of reproductive isolation through spyglass starts late when speciation already completed, and looks back in time. This approach focuses on postzygotic genetic incompatibilities of hybrid sterility or inviability and allopatric speciation remains the null model.

2) Prospective analysis in early stage of speciation starts early through magnifying glass when species born. It looks perspective or prospective in time how a variable population evolves into a divergent pair of populations. This population level analysis focuses on how ecological, sexual and social selection pressures and genetics interact and cause the evolution of sexual or ecological barriers to gene flow and how this result in partial reproductive isolation.

Top-down and bottom-up ways to study the genetic and structural changes in the selection processes of speciation have been outlined (Andersson & Simmons 2006) Top-down inferring causes from phenotypic pattern and bottom-up from DNA sequences via protein to phenotypic expression. Top-down is a deductive reasoning approach with theory-driven method and bottom-up is an inductive approach with data-driven method. The resource intensive multidisciplinary models of top-down (morphology-molecules) and bottom-up (molecules-morphology) speciation research are resource limited. The pure taxonomic bottom-up model from fine structure to gross morphology is resource effective. It may help us to find initial split criterion in order to understand the early stages of speciation as well as the real diverging point of a newly born species.

**Pressures in accelerated speciation**

Natural, ecological, sexual, and social selections are different forms of the same process with interrelations. A possible alternative idea is to consider the ecological, sexual and social environment as stimulating and acting pressures in natural selection. Understanding how reproductive barriers evolve during accelerated speciation remains an important question in evolution. Divergence in mating preferences may be a common first step in this process.

Ecological speciation is defined as the evolution of reproductive isolation through ecologically based divergent natural selection. Reproductive character displacement by reinforcement may play a diversifying role when previously allopatric populations join. Divergence in sympathy may be driven by sexual conflict or by association of mating types with ecological differences. In a broad definition of sexual selection, traits that influence competition for mates are sexually selected, whereas those that directly influence fecundity or offspring survival are naturally selected.

**Combined sexual and ecological selection as mainspring of diversification.** Mate choice by phenotype matching cannot be hindered by recombination because the same genes control both the mate signals and the mate preferences (Conte & Schluter 2013). Speciation is made even faster if phenotype matching is based on a trait under divergent natural selection. In this case, assortative mating should rapidly evolve as a byproduct of divergent selection on the trait. Sexual selection is strong and rapid especially in conjunction with ecological divergence (Bonduriansky 2011). Rapidly changing environment can drive and speed reproductive isolation in the process of ecological speciation. During adaptive radiation the speciation rates are accelerated by the availability and diversity of the newly exposed resources. Later niche filling and resource limitation decelerate speciation rates. Ecological differences can drive the evolution of partial rep-
reductive barriers in dozens to hundreds of generations (Hendry et al. 2007). Some exceptionally rapid pleistocene speciation probably followed similar temporal cycles (Phillimore & Price 2009).

The postcopulatory cryptic female choice is achieved during the long temporal and spatial journeys of the sperms within the female genital system. This sexual selection mechanism with various female-controlled processes is more distributed, particularly in polyandry, than the classic Darwinian precopulatory female choice (Eberhard 2009, 2010). Promiscuous females that mate to both related and unrelated males are able to bias paternity against their relatives to avoid inbreeding. They control and manipulate sperm storage in the process of cryptic female choice (Bretman et al. 2009).

Gamete recognition. Fertilization is realised by the interaction of proteins on the surfaces of sperm and egg. These proteins may evolve rapidly and supported by definitive block to polyspermy.

Accelerated ways of speciation

There are no universal molecular clocks for invertebrates even if we apply the „relaxed clock” method recalibrated for variable substitution rates. The accumulation process of fixed mutation is very complex (Thomas et al. 2006). The genetic distance measured by DNA sequence analysis is related to lineage divergence time but with unconstant rates. The speciation speed estimated by molecular clock is in the range of million years. However animal and plant communities in many parts of the world have shorter history. For instance the environmental alteration at the end of the Pleistocene, only 10,000 years ago, triggered rapid evolution of juvenile incipient phylogenetic species. Genetic models of adaptation created by Modern Synthesis are focusing on allelic variation, Mendelian inheritance and random gene mutation with slow speciation processes. These former models are incomplete and unable to explain mechanisms of the accelerated divergence processes under various selection pressures. Today there is growing diversity of mechanisms allowing inheritance of acquired traits. It seems that heredity is not mediated by a single, universal mechanism. A pluralistic model of heredity is now emerging (Bonduriansky 2012). Newly discovered mechanisms can accelerate or even modify the rate and direction of adaptation in various speciation pathways.

Genetic inheritance. Speciation can be rapid under both ecological and mutation-order models, because alleles are driven to fixation by natural selection in both cases. However mutation-order speciation is more difficult when gene flow spreads favourable mutations to other populations, preventing divergence (Schluter 2009). Migration and also the genetic drift affect the entire genome, but the effects of natural selection are limited to the genomic regions harboring loci that affect the selected phenotypic trait. Ecological selection maintains divergence in those parts of the genome that affect favorable traits, while gene flow continues on other genomic region. Therefore in early stages of ecological speciation with gene flow (in sympatry) the genetic divergence is restricted to the divergently selected genomic region of the favorable quantitative trait loci. In incipient species this genetic mosaic of speciation involves only few stable characters of the diverging loci leaving the genom largely homogenised with polymorphisms by ongoing gene flow. These noise or random variations are neutral changes with respect to selection. Nearby the diverging loci the selective sweep reduces polymorphism through genetic drift or evolutionary hitchhiking. In rapidly adapting populations, the loci with abnormally high outlier values are under divergent selection and its split could be estimated by retrospective coalescent simulation.

Nongenetic inheritance. Inheritance mediated by the transmission to offspring of elements of the parental phenotype or environment: epigenetic state (epiallele), cytoplasmic and somatic factors, nutrients, extraorganismal environment, behavior, and culture (Bonduriansky & Day 2009). According to hard/soft dichotomy the hard heredity is the Central Dogma of the exclusive one-way passage
of information from DNA sequence to RNA to protein, refuting the possibility of soft or Lamarckian heredity that an individual’s experiences during its lifetime could have predictable effects on the phenotype of its offspring. Nongenetic inheritance can track too rapid environmental changes for genetically based adaptation. It can overcome some of the limitations of genetic inheritance, partially decoupling phenotypic change from genotypic change by using acquired traits, by permitting transmission of favorable trait combinations when genetic recombination is diluting its pool, by generating heritable phenotypic variation to replace the depleted additive genetic variation (Bonduriansky 2012).

**Genetic encoding.** In nongenetic inheritance a hypothesized process whereby an acquired trait is encoded in the germ-line DNA sequence, thereby giving rise to a new, transmissible gene allele (Bonduriansky 2012).

**Biased mutation.** Speciation processes could be speeded up by non-random mutation, when particular environmental factors tend to induce particular changes in the DNA sequence (Bonduriansky 2012). Some non-random *epimutation* are also influenced by environment. Similar to *nucleotide mutations*, *epimutations* have the potential to be beneficial, neutral or deleterious.

**Developmental phenotype plasticity.** Within-generation and transgenerational phenotypic plasticity bring individuals closer to phenotypic optimum allowing populations to persist through periods of rapid environmental change when slow genotypic changes cannot keep pace during evolution sensu stricto (Bonduriansky et al. 2012). Natural selection acts upon favorable mutation, but this random process is unlikely to produce all the variants therefore the importance of environmental induction in evolution should not be ignored.

**Phenotypic and genetic accommodation.** Baldwin effect (Baldwin 1896) is based on two concepts of rapid changes.

1. **Organic selection** is the ability of plasticity to increase survival.

2. **Orthoplasy** is the directional influence of organic selection on evolution.

Plastic individuals are able to adapt to environmental changes rapidly within one generation. This plasticity dictates the course and direction of evolution and over time standing genetic variation can be selected in the direction of induced plastic response. Baldwin spoke of accommodation in reference to this non-heritable phenotypic change. Today (Crispo 2007) we distinguish phenotypic accommodation (Baldwin’s accommodation) and genetic accommodation when heritable variation occurs in the same direction as the plastic response, similarly to Baldwin’s coincident variation or orthoplasy.

**Genetic assimilation** (Waddington, 1942) suggests that environmentally induced phenotypes may become genetically fixed (Pigliucci & Murren 2003), the induced phenotypic variation becomes constitutively produced (Pigliucci et al. 2006) and no longer requires the original environmental stimulus for expression. In the original idea of *phenocopy* (Goldschmidt 1940) the environmentally induced phenotype looks like the result of a genetic random mutation.

**Epigenetics.** Rapid heritable changes, phenotypic variations in gene expression are frequently unexplained by differences in DNA sequence. Epigenetic fitness differences, as selection pressures provide additional system of heritable variation for natural selection. Epigenetic variations, unlike genetic variations are alterable directly by ecological or sexual interactions, providing accelerated way for evolutionary change (Bossdorf et al. 2008). Epigenetic changes are based on molecular processes like DNA methylation or chromatin acetylation and methylation. Epigenetic processes modify genotype expression through epigenotype to phenotype. Transgenerational epigenetic inheritance supports Lamarckian inheritance of acquired phenotypic traits when environment in one generation can cause epigenetic changes that are inherited for multiple generations. Acquired trait could be encoded in the germ-line DNA sequence initiating new, transmissible gene allele.
Phenotypic trait variability

In alpha taxonomy we have to find stable trait for initial split criterion to separate juvenile incipient taxa when applying the phylogenetic species concept. In nature the intraspecific trait variation occupies a central role as rough material for evolutionary sorting processes of natural selection and random drift. The description of a single individual is frequently not sufficient to describe an entire species. No two individuals of the same species are identical. Even identical twins, possessing the same set of genes, have different fine structures due to the random effects of any biological processes. Instead, the description of many individuals taken together defines a range of variation that encompasses the species.

Different individuals possess different sets of genes forming the intraspecific variation (Darwin’s individual variability). The observable individual variability is referred to as phenotypic variation or as phenotypic polymorphism. In alpha taxonomy we search stable specific characters of phenotype with trials to understand the range of phenotypic variation. Phenotypic variation results from genetic and environmental factors. The genetically controlled phenotypic variation is recognised as genetic polymorphism or simple polymorphism. There are three primary sources of genetic variation:

1. **Mutations** are changes of the nucleotide sequence of the genome. A single mutation can have a large effect, but evolutionary change is based on the accumulation of many mutations.
2. **Gene flow** is any movement of genes from one population to another.
3. **Sex** can introduce new gene combinations into a population by genetic reshuffling (recombination) and by genetic random drift. **Standing genetic variation** represents alleles already present in population.

Phenotypic variability is the tendency or potential of an organism to vary (Wagnes & Altenberg 1996), while phenotypic variation can be observed and documented. Variability represents a range of potential outcomes and expressed phenotypic variation in a population what is available to natural selection. Developmental processes and their interactions limit the variability including molecular, genetic, cellular, individual, population and environmental factors. Development itself is evolvable with interactions producing ever-changing landscape of variability. Processes of canalization, developmental stability and morphological integration are interrelated components of variability (Willmore et al. 2007):

1. **Canalization** ensures similar phenotypic expression buffering development against both environmental and genetic perturbations. Canalization is measured by pattern and amount of among-individual variation indicates differences in ability to canalize development against genetic and environmental stresses.
2. **Developmental stability** ensures consistent phenotypic expression within individuals and measured by within individual variation or fluctuating asymmetry. Any deviation from symmetry reflects some developmental instability, high levels of fluctuating asymmetry indicate low level of developmental stability. Both developmental stability and canalization limit the expression of phenotypic variation, but differ according to how they are measured.
3. **Morphological integration** refers to the phenotypic interdependence of two or more structures enhancing the overall stability of the organism. Pleiotropy and linkage disequilibrium create genetic integration and coordinated evolution of traits is considered as evolutionary integration. Modularity is related to the concept of morphological integration and the module is a set of characters integrated internally. Morphological integration is estimated by measuring the level of covariation or correlation among structures.

Even subtle structural variations seem to be correlated with fitness parameters in local specialization for resource use. Morphological differentiation should be reduced when individuals shift from a geographically heterogeneous habitat. **Adaptive variation** suggests that morphologies of population members can result in differences in their niches. The **niche variation hypothesis** (Van Valen 1965) suggests that populations with wider
niches (generalist) are more variable than populations with narrower niches (specialist). Phenotypic variation in a generalist population could be achieved by an increase in genetic variation and by phenotypic plasticity if plasticity itself is evolvable. Constraints on genetic variation, such as the absence of assortative mating, may limit the amount of variation that can evolve in a sexual population. Response to selection in the early stages of divergence is based on standing genetic variation, producing more rapid speciation than the variation generated from new mutation.

MATERIAL AND METHODS

Cooperation to put together available materials

The discovered Potamophylax nigricornis species group could be a deterrent depressing model how we miss to resolve the fine diversity of our living world even in the “intensively studied Europe”. In spite of the ongoing declared “green” policy the science of biodiversity, the basic science of ecosystem services, that is the alpha taxonomy is resource limited, not supported. To overcome this mismanaged environmental policy the first author has developed an idea of cooperation how to realise comprehensive studies on the so called obscured taxa. Many European caddisfly species with wide distribution and with high apparent phenotypic variability are possible object of such cooperation. Many of these noise or random variations are neutral changes with respect to selection and frequently accompanied by stable traits, the product of the particular selection during the early stages of speciation. These childhood stable traits of the incipient phylogenetic species could serve as reliable initial split criteria to distinguish a newly evolved species. The neutral random variations in the populations of the so called widely distributed and highly varying “species” are the direct signs of intense speciation processes. This can be demonstrated if we are able to confirm the particular selection process by finding stable morphological traits, the target of selection and the first morphological product of speciation.

We have already documented such condition, under the pressure of sexual selection at Chaetopteryx rugulosa species group. In this group the periphalline organs exhibit high variation, but some structures on the intromittent phallic organ are very stable (Oláh et al. 2012). It is promising that FSA offers us to find such a stable characters. These early products of selection are not yet confounded by additional differences. These additional diversifying structural changes, mostly among the periphalline organs develop later in the adult stages of the species. In FSA we need to examine many specimens from many populations. Today under the present course of resource disposing policy we have to rely upon caddisfly specimens already collected in various research projects and deposited in various collections. If money limits our efforts in alpha taxonomy we have to put together what we have. To bring together these scattered specimens we need a specialist interested in that particular species complex. He will initiate and organise this collective effort. We understand that collected, sorted and determined material incorporates already significant scientific work and has high primary value for such surveys. Therefore we practice that colleagues who contribute to the survey with their specimens and agree with the final findings become coauthor of the paper and/or of the species automatically.

Fine Structure Analysis (FSA)

Early steps of divergence can be studied by magnifying glass and by bottom-up procedures. Both approaches make it possible to find initial split criteria before they become confounded by additional differences. This population level bottom-up research by “magnifying glass” can be realized either by sophisticated and expensive molecular genetics or by simple and cheap FSA. Boths are particularly suitable to analyse speciation processes under sexual and ecological selection pressures. We have already documented that bottom-up magnifying glass of FSA may cope effectively with the challenge to find the initial split criterion in alpha taxonomy (Oláh et al. 2012, Oláh & Ito 2013). This pinpoint precision
helps us to recognise the phylogenetic species along a continuum of divergence. The FSA is directed to determine the stability and variability of phallic organ and/or vaginal sclerite complex. Finding stable fine structures nearby the structural diverging point requires high structural resolution and patient effort to determine the ranges of phenotypic variations. Studies on stability and variability are possible if we have many specimens from many populations as well as if we apply careful clearing (chemical) and cleaning (mechanical) processes. It may well happen reasonable that a new phylogenetic juvenile species is described from a single male or female or from a few specimens. In this case future studies will confirm the stabilities of the putative specific traits.

FSA is also applicable to estimate divergence distance between incipient species. Like in molecular clock the structural distance is related to lineage divergence time. In order to quantify a structural clock this pure phenomenological relation could be calibrated by known absolute age of evolutionary divergence events, geological event or by fossils.

**Limits of Initial Split Criterion (ISC)**

The theory of morphological initial split criterion is formulated to detect the initial separation or the early divergence of the ancestral lineage. It has some analogy to the retrospective model of coalescent theory in genetics tracing the ancestry of two taxa back to the most recent common ancestor. In principle, applying FSA, we are able to find any stable morphological divergent trait evolved either in males or in females. In practice the primary premise when applying FSA for ISC is that a single individual never sufficient to describe a species. We need to examine many specimens of males and females in many populations in order to determine the range of phenotypic variation. The description and drawings of divergent trait of several specimens represent the specific morphological range of the phenotypic variation. Nevertheless the description based on a single individual could be an important taxonomic action to initiate, inspire, or provoke further morphological or molecular studies. Having only single male specimen we have described *Potamophylax peremes* sp. nov. from Italy and the subspecies of *Potamophylax nigricornis testaceus* (Zetterstedt, 1840) was raised to specific rank as *Potamophylax testaceus* (Zetterstedt) stat. nov.

**Clearing, cleaning and drawing procedures**

This study is based on animals preserved in 70–80% alcohol. In order to observe morphological details in the genitalia, the entire or only the terminal segments of abdomen were removed and placed in a small glass beaker of 25 cm³ with 10% KOH solution and boiled during 5–15 minutes for digestion above a spirit burner. The duration of the treatment is adjusted individually to the effectiveness of clearing process which depends on the species or even on the nutritive state of tissues or on the physiological condition of the specimens. The process of digestion can be easily followed by transparency. The dissolution rate of the soft tissues, the clearing transparency is visible to naked eye. The clearing process and time are so much taxon, size, age, sex, and nutrition state specific that automatic hot plate or bath clearing is not practical. The digested abdomen was subsequently transferred to distilled water and the macerated tissue was removed mechanically in patient cleaning process by fine tipped forceps and needles. The internal vaginal sclerite complex was exposed to clear view by cutting windows into the dorsum and left pleuron with fine scissor. The cleared and cleaned abdomen was transferred to 80% ethyl alcohol, and to glycerine for examination under microscope. Different sized pins modified to supporting ring bottom was introduced into the abdomen and used to hold and stabilise the genitalia in lateral, dorsal, and ventral position for drawing. However, the plane of view is never perfect and we made no special procedures of grid, matrix, or reflection to produce absolute mirror symmetry of the drawings. Instead, the genital structures are drawn exactly as seen in the microscope. However setae are represented only by their alveoli and moreover their density is only symbolic. If essential the
setal length or setal shape are presented by drawing a single or a few setae only. The genital structure was traced by pencil on white paper using a drawing tube mounted on a WILD M3Z microscope at between 260x and 416x magnification. Final illustrations were prepared by enlarging the original pencil drawings and redrawn on transparent paper by Black India Ink.

**Terminology**

We used our functional appendicular terminology and not the conventional anatomical directional terminology to describe the genital structures in species description (Oláh & Johanson 2008). Species descriptions were standardized to ensure consistently formatted and comparable description in general accord with Evenhuis's template principle (2007). We have standardized also the terminology to describe space extensions of variously formed structural elements. The following terms were used to qualify the dimensions and extensions of genital structural elements:

1. *short or long* for length dimension on the longitudinal direction of coronal plane along the anteroposterior axis;
2. *low or high* (traditionally shallow or deep especially for excisions) for height dimension on the vertical direction of the sagittal plane along the dorsoventral axis and
3. *narrow or wide* (broad) on the lateral direction of the transversal plane along the mediolateral or left-right axis. The three dimensional Cartesian coordinate system provides theoretical possibility to quantify by measurements the three physical dimensions of length, width, and height of each structural element. However this quantification is used very seldom in species description. Here we quantify only the length of forewing.

**Variability of the phallic organ**

Phallic organ is composed of phallobase, aedeagus and paramere. Phallobase (phallic apodeme+phallotheca+endotheca) starts with a very short ringlike phallic apodeme fused to and continuing with the sclerotized tubelike phallotheca that housing the retracted membranous endotheca. The retractable and erectile endotheca holds the aedeagus and parameres. The phallobase suspended on its dorsoapical rim by a pair of sclerotized straps. These straps are located dorsolaterally connecting the phallobase to the area where the basal triangle of the paraproct and the finger-like sclerotized strip of segment IX meet. The three meeting structures seem not fused sclerotically. The sclerotized aedeagus forms bifid apex and ventral subapical variously developed heels. Membranous retractable endophallus with the gonopore is nested in the dorsal depression of the aedeagus. Its main function is to direct and fit the gonopore to the opening of the spermathecal process. Gonopore configuration is variously sclerotized, ejaculatory duct discernible. Pair of parameres is rooted in the endophallus and composed of well developed shaft and spine-like setae of various and specific numbers and patterns. These strong spine-like structures articulate to the shaft with alveoli.

The sclerotized stem of the aedeagus has specific ventral profile with diagnostic value that could be parallel-sided or variously bellied laterad. The ventral subapical heels on the aedeagus head have evolved specifically into various forms that have high diagnostic value, in spite of certain variability in the range of specificity. The very tip of the bifid aedeagus supplied with very small setae of sensory function inside the female genital chamber. This tip is rather variable within population. It is possible that tips are erectile and the apparent variability what we detect is the result of erection stage. Other alternative is the unusually high phenotypic variability.

The dorsal and lateral profile of the paramere shaft including its sclerotized basement is rather stable. Even more stable is the pattern of the spine-like setae, having very high diagnostic value. These setae have stimulatory function during copulation giving signals of various functions to meet the female preference range in the processes of cryptic female choice of sexual selection. Frequently it is not easy to visualise the genuine pattern due to their disturbed or injured
condition. During copulation the spine-like setae are exposed to various effects resulting in deformation, or even in breaking down at alveoli or at various lengths. Usually the original virgin setal pattern is very disturbed after copulation, discouraging the observers.

**Structure and function of vaginal sclerite complex**

The diversity potential of the sclerotized structure functioning in the female genital chamber is underutilized in distinguishing among the closely related caddisfly species. Female internal apparatus cleared in caustic potash was first recognised and applied by Morton (1902), later by Nielsen (1943), to separate *Apatania* females. In limnephilids the vulvar opening formed and surrounded by the lower lip (*vulvar scale* of McLachlan (1974–1880), *gonopods of segments VIII and IX* by Nielsen (1980)) and by the upper lip (*supra-genital plate*, part of segment X) is the vestibule to vagina. The vaginal chamber is formed by fusion of the distal parts of the common oviduct and the duct of the accessory or collateral glands. These glands usually are very large filling most part of the female abdomen and their ducts are rather wide at their opening section. This may divide the vaginal chamber into a ventral and dorsal branch. The *vaginal sclerite complex* (*internal sclerite* of Morton (1902), *spermathecal sclerite* of Nielsen (1980)) developed along the junction of oviduct and the duct of the accessory glands and receiving also the spermathecal duct plus the duct of bursa copulatrix. It is a rather diverse and complex organ, but this potential was not yet explored to differentiate among caddisfly species. Species specificity of female genitalia, higher than at male, was demonstrated only recently in families of dipteran Sepsidae (Puniamoorthy et al. 2010) and mecopteran Panorpidae (Ma et al. 2012). Its complex nature as well as difficulties in understanding and drawing, limited its use in taxonomy. We understood the vaginal sclerite complex evolved with flexing, bracing, holding, and stretching functions for the structural organisation of the four ducts entering and forming the vaginal chamber. Its dorsal position to oviduct and anterad position to the duct of accessory gland as well as the variously developed sclerotized substructures to receive duct of bursa copulatrix and the duct of spermatheca explain this basic function. Morton’s original terminology is still rather functional. Based upon his original findings, we have differentiated six substructures in the vaginal sclerite complex for our taxonomic purposes.

1. Morton’s *paired lateral blades* are the *vaginal sclerite plate* itself on the dorsum of the vagina. The vaginal sclerite plate may form variously sclerotized lateral folds, flanks and subdivided structures in different groups. We have separated two additional substructures of the plate with particular functions.

2. The substructure of mostly sclerotic articulation to the internal continuation of the lateral processes of the vulvar scales, the paired gonopods of segment IX, is usually a double layered *folding plica* ensuring a firm flexible attachment or suspension of the membranous genital chamber and its tubing complex to the exoskeleton of the vulvar scale.

3. The vaginal sclerite plate has a pair of sclerotized wing-shaped substructure lateral serving stretch function to the vagina and apodemic function anterad to receive vaginal muscles.

4. Morton’s *central triangular piece* is the *usually hood-shaped junction sclerite* holding and stretching the junction where the ducts of ovarium and accessory gland meet.

5. Morton’s *central foot-shaped piece* is the *spermathecal process* (*processus spermathecae* of Nielsen (1980)) receiving the ductus spermathecae and forming frequently a longitudinal keel on the ventrum of the vaginal sclerite. The opening of the spermathecal duct forms variously sclerotized window on the spermathecal process.

6. This small sclerite was not specified by Morton. The ductus bursae open between the spermathecal process and the common oviduct at the anterior margin of the vaginal sclerite. The mesoanterior margin of the vaginal sclerite plate is bulking and bending upwards elevating the position of the duct opening. These substructures and functions constitute the vaginal sclerite complex, but their development and sclerotization are highly varying in the different groups.
Windows to examine and to draw the vaginal sclerite complex

The carefully cut dorsal and lateral windows on segment VIII give a clear view for the examination and drawing of internal vaginal sclerite complex in general. In the *Potamophylax nigricornis* species group the vaginal sclerite complex incorporates several morphological informations discernible both in dorsal and lateral view. In the present species descriptions we utilise only the dorsal profile of this complex structure to differentiate between species. This dorsal profile is rather simple, but also rather stable and specific. The lateral view produces more morphological informations, but this view is extremely sensitive to the observation plane. It is almost impossible to reproduce the repeated redrawings. Various folding, curving and bending organisations in three dimensions create very composite and complex structure composed of the six substructures: lateral margins of the vaginal plate, the articulation sclerite, the wing-shaped vaginal stretching plate, junction sclerite, spermathecal process, bursal sclerite. This very composite structure contains more unexplored specific informations to find initial split criteria with a more detailed finer structure analysis.

**Depositories**

Civic Natural Science Museum, Bergamo (CNSMB)
Coppa Private Collection (CPC)
Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina, Prishtina, Kosovo (DBFMNSUP)
Hungarian Natural History Museum, Budapest (HNHM)
University Museum of Bergen, University of Bergen, Norway (ZMBN)
National Museum of Natural History, Bulgarian Academy of Sciences (NMNH BAS)
National Museum, Prague, Czech Republic (NMPC)
Oláh Private Collection (OPC) under national protection of the Hungarian Natural History Museum, Budapest
Sipahiler Collection in the Department of Biology Education, Hacettepe University, Ankara, Turkey (SCHUA)

**TAXONOMY**

*Potamophylax nigricornis* group

This new species group is composed of closely related species characterized by rather stable periphallic organs, but very diverse phallic organ. The periphallic organ consists of long digitate paraprocts and gonopods as well as the more or less elongated cerci. The long paraprocts and the acute gonopods (McLachlan 1874–1880) serve best to separate this species group from allied taxa. The widely distributed nominate *Potamophylax nigricornis* species is possibly the ancestor of the entire species group. At least it has a wide distribution and the evolved species seem all peripatric. Moreover it has the most complex paramere and according to Williston’s principle the structures tend toward reduction. Therefore an ancestor must be constituted by the integration of the largest possible number of characters (Schmid 1979). Although we have to remind that the terms primitive, generalized, specialized, simple, complex or secondarily complex are all strictly comparative (Ross 1956; Schmid 1958).

The ancestral paramere of *P. nigricornis* is characterized by sigmoid shaft with subquadratic basement in dorsal view, as well as by complex setal pattern with basal tuft of 5 mesad curving long spine-like setae accompanied by 2 regularly set subapical and 2 apical spine-like setae. This sophisticated structure is extraordinary stable in the examined 106 populations over the entire distributional area. The discovered new species evolved probably from this dark species having this very complex paramere. Diversification developed possibly during the Pleistocene mostly in peripatry and probably in the sexual selection processes. The speciation processes are detectable by depigmentation and by the simplification of the parameres and by the modifications of the aedegal head. Based on these structural changes we have separated an ancestral and three descendant species subgroups simply for taxonomic practices: *Potamophylax nigricornis* new species subgroup, *P. elegantulus* new species subgroup, *P. horgos* new species subgroup, *P. simas* new species subgroup. However it seems, but not examined here,
that their speciation was associated with more than one glacial-interglacial cycle at least according to the distribution pattern and detectable also by the branching structure of lineages among the described species. Branching is discernible along the depigmentation pattern, paramere simplification, and aedeagal modification.

**Potamophylax nigricornis new species subgroup**

Forewing patterned by narrow, longitudinal pale stripes present in the cells on the dark forewing background. Paramere characterized by sigmoid shaft with quadratic basement as well as by complex setal pattern with basal tuft of 5 mesad curving long spine-like setae accompanied by 2 regularly set subapical and 2 apical spine-like setae. The ventral subapical heel on the aedeagus present as variously developed pointed corner plate. Two species belong to this ancestral subgroup: the nominate species *P. nigricornis* and the incipient, just diverging species *P. testaceus* Zetterstedt status novus.

**Potamophylax nigricornis (Pictet, 1834)**

(Figures 1–6)

*Phryganea nigricornis* Pictet, 1834:136–137, (Switzerland).


**Diagnosis.** This widely distributed, probably ancestral species has dark gray forewing with narrow longitudinal pale stripes present in the cells on the dark forewing background. Paramere characterized by sigmoid shaft with quadratic basement as well as by complex setal pattern produced by basal tuft of 5 mesad curving long spine-like setae accompanied by 2 regularly set subapical and 2 apical spine-like setae. The ventral subapical heel on the aedeagus as pointed corner plate with variously developed tooth-like profile.

1984, light trap, (1 female, OPC). Sopron, 8.VI.
1984, light trap, (1 male, OPC). Sopron, 19.V.
Velem, Borha spring, 23.VI. 1987, leg. S. Nógrádi
& Á. Uherkovich, (6 males, 6 females; OPC).
Mecsek Mts. Kisújbánya, Pásztor spring, 18.VII.
1984, leg. A. Uherkovich, (2 males, 2 females;
VI.1988, leg. A. Uherkovich, (2 males, OPC).
Mecsek Mts. Vékény, Vár valley, Iharos spring,
20.VI.1994, leg. S. Nógrádi & Á. Uherkovich,
(4 males, OPC). Mátra Mts. Gyöngyös-Mátrafüred,
Waterworks, 2.VII.1987, leg. F. Buscgmam, (2
males, OPC). Bükk Mts. Garadna stream, 8.VII.
1983, light, leg. J. Oláh, (3 males, OPC). Jósvafő,
VIII.1980 light, leg. Z. Varga (10 males, 9 fe-
males; OPC). Jósvafő, VII.1983 light, leg. Z. Varga
(1 male, OPC). Jósvafő, VIII.1981 light, leg. Z. Varga
(3 males, OPC). Jósvafő, VIII.1982 light, leg. Z.
Varga (2 males, OPC). Jósvafő, V.1981 light, leg. Z.
Varga (2 males, OPC). Jósvafő, VI.1981 light
leg. Z. Varga (14 males, 6 females; OPC).
Jósvafő, 1–2.VI.1981 light, leg. Z. Varga (1 male,
OPC). Jósvafő, VIII.1981 light, leg. Z. Varga
(3 males, OPC). Jósvafő, 3–9.X.1981 light, leg. Z.
Z. Varga (1 male, OPC). Jósvafő, 5–10.VI.1982,
light leg. Z. Varga (2 males, 1 female; OPC).
Jósvafő, 10–11.VI.1982 light, leg. Z. Varga
light, leg. Z. Varga (2 males, 1 female; OPC).
Jósvafő, 22.VII.1982 light, leg. Z. Varga (1 male,
OPC). Jósvafő, 1–2. VIII.1982 light, leg. Z.
Varga (1 male, OPC). Jósvafő, 14–15.VIII.1982 light,
VIII.1982 light, leg. Z. Varga (2 males, 1 female;
OPC). Jósvafő, 7.X.1982 light, leg. Z. Varga
(1 male, OPC). Jósvafő, Ménsvölgy, Ménét stream,
spring stream, 3.VII.1983 singled, leg. J. Oláh
(1 male, OPC). Jósvafő, Ménsvölgy, Patkós spring,
4.VII.1983 singled, leg. J. Oláh (1 male, OPC).
Jósvafő, Ménsvölgy, 5.VII.1983 light, leg. Z.
Varga (4 males, 1 female; OPC). Jósvafő, 5–10.
VI.1983 light, leg. Z. Varga (9 males, 17 females,
OPC). Jósvafő, 18.VI.1983 light, leg. Z. Varga
(18 males, 17 females; OPC). Jósvafő, 28–29. VI.
1983 light, leg. Z. Varga (14 males, 10 females;
OPC). Jósvafő, 30.VI.1983 light, leg. Z. Varga
(1 male, 3 females; OPC). Jósvafő, 2.VII.1983 light,
leg. Z. Varga (2 males, 3 females; OPC). Jósvafő,
4–5.VII.1983 light, leg. Z. Varga (1 male, 2 fe-
males; OPC). Jósvafő, 6.VII.1983 light, leg. Z.
Varga (1 female, OPC). Jósvafő, 1.VII.1983 light,
leg. Z. Varga (1 female; OPC). Jósvafő, 7–9.VII.
1983 light, leg. Z. Varga (3 males, 2 females;
(14 males, 3 females; OPC). Jósvafő, 9–31.V.
1983 light, leg. Z. Varga (5 males, OPC). Jósvafő,
Jósvafő, V.1983 light, leg. Z. Varga (6 males, 4
females; OPC). Jósvafő, Tohonya völgy, 10–12.
VII.1983 leg. Z. Varga (3 males, OPC). Jósvafő,
Tohonya völgy, 5.V.1984 leg. Z. Varga (2 males,
Z. Varga (6 males, OPC). Jósvafő, VIII.1985 light
(14 males, 3 females; OPC). Jósvafő, 13.V.1985
light, (1 male, 2 females; OPC). Jósvafő, 13.VI.
1985 light, leg. Z. Varga (6 males, 9 females;
OPC). Jósvafő, Tohonya völgy, 20.V.1986 leg. Z.
Varga (3 males, 4 females; OPC). Jósvafő, Toho-
yoa völgy, 26.V.1986 leg. J. Oláh (6 males, 4
females; OPC). Jósvafő, Tohonya völgy, 14–16.
VI.1986 leg. J. Oláh (2 males, 4 females; OPC).
Szőliget, 20–30.V.1986 light trap (19 males, 4
females; OPC). Szelcepuszta, 15.VIII.1982 light,
(1 male, OPC). Szelcepuszta, V.1983 light, (2
light, (1 male, OPC). Zemplén Mts. Telkibánya,
Telkibánya, 25.VIII.1984 light, (1 male, OPC).
Zemplén Mts. Telkibánya, 1–2.IX.1984 light, (1
male, OPC). Zemplén Mts. Lászlótanya, 2.X.
1982 light, (1 male, OPC). Zemplén Mts. László-
tanya, 5.X.1982 light, (1 male, OPC). Zemplén
Mts. Lászlótanya, V.1983 light, (5 males, 1 fe-
males; OPC). Zemplén Mts. Lászlótanya, VII.
1983, (18 males, OPC). Zemplén Mts. László-
J. Oláh (31 males, 43 females; OPC). Zemplén
light, (1 male, OPC). Zemplén Mts. Lászlótanya,
14.VI.1985 light, leg. J. Oláh (31 males, 43 females;
Zemplén Mts. Füzér, Lászlótanya, 15.VI.1995
leg. V. G. Papp (1 male, 1 female; OPC). Zemplén
Mts. Makoshotyka, 27.IX.1982 light, (2
IX.1983 light, (4 males, 1 female; OPC). Zemplén
Mts. Makoshotyka, 16.IV.1984 light, (2 males,
1 female; OPC). Italy: Lombardia, Bergamo, Gaz-
zaniga, Valle Platz, 850 m, 27.V.1990 leg. C.
Gusmini (1 male, CNSMB). Lombardia, Berga-
mo, Tarvisio, Rio del Lago, N46.4882 E13.6724,
870 m, 21.VII.1996 leg. C. P. Pantini & M. Valle
(1 male, CNSMB). Friuli-Venezia Giulia-Udine,
Lusevera, torrente Vedronza, N46.2609 E
13.2567, 330 m, 21.VII.1996 light leg. C. P. Pan-


Romania: Transylvania, Valea Cupas, Lacu Rosu, 950 m, 17.VII.1981, light leg. Peregovits & Ronkay (1 male, HNHM). Transylvania, Valea Cupas, Lacu Rosu, 950 m, 19.VII.1981, light leg. Peregovits & Ronkay (2 males, HNHM). Maramures county, Maramuresului Basin, Sighetu Marmatiei, Mocsár area, orchard, N47°55’07.1’’ E23°56’43.5’’, 369 m, 30.VI.2005 leg. J. Kontschán, D. Murányi & K. Orci (1 male, NHMB). Maramureș county, Muntii Ignis, Desești-Stația Izvoare, forest spring at settlement, 920 m, N47°45’11’’ E23°42’58’’, 8.VIII.2012 light, leg. J. Oláh & L. Szél (7 males, 5 females; OPC). Maramures county, Maramures Mts. Frumuseaua stream, 764 m, N47°52’43’’ E24°18’22’’, 7.VIII.2012 light, leg. J. Oláh & L. Szél (1 male, 1 female; OPC). Slovakia: deposited in NMPC under K263 with label: Worochta, Stoeckel. Vorokhta is a settlement on northern slopes of the Ukrainian Carpathians (locality, where also Dziedzielewicz collected). The collecting date is missing (probably the end of 19th century). This species is from the Klapalek’s collection, but it is not a part of series of Potamophylax nigricornis v. elegantulus.

Variability. Both the head structure of the aeradus as well as the shaft and setal pattern of the parameres are remarkably stable in all of the examined 106 populations representing specimens from Austria, Croatia, Czech Republic, Hungary, Italy, Norway, Poland, Romania, Slovakia, Slovenia, Ukraine. Only the ventral subapical heel, that is the variously formed, pointed pair of corner plate reduced in size in 4 specimens from two populations: Czech Republic (Bohemia, Jizerské hory), Hungary (Szelcepuszta). However even in these specimens the very characteristic narrowly S-shaped shaft and the setal pattern are very stable.
Figures 1-6. *Potamophylax nigricornis* (Pictet, 1834) male. 1 = genitalia in left lateral view, 2 = genitalia in caudal view, 3 = phallic organ in ventral view, 4 = paramere in dorsal view, 5 = phallic organ in left lateral view, 6 = aedeagus in ventral view. (Figures 1–3 were drawn by the first author 50 years ago in 1963 with some features of habitus drawings; figures 4–6 are drawn recently in diagrammatic styles).
Potamophylax testaceus (Zetterstedt, 1840) stat. nov.
(Figures 7–9)

Phryganea testacea Zetterstedt, 1840:1065-1066. Type specimens are not available!
Stenophylax nigricornis var. testaceus (Zetterstedt, 1840), Brauer, 1876:286.
Potamophylax nigricornis (Pictet, 1834), Transferred and listed in genus Potamophylax by Schmid 1955:176.

Diagnosis. This light coloured taxon has pale lines on the forewings almost obsolete. Compared to P. nigricornis the forewing colour is testaceus, brick red, not gray mouse; the pointed corner plate on the ventral subapical heel on the aedegus reduced in size; lateral bellies on the aedeagus very much developed, not moderate; subquadratic basement of the shaft enlarged; single spine-like apical seta present, not two. All these distinguishing characters were established by the examination of the available single male specimen. The ranges of phenotypic variations has to be examined and the stability of these diverging characters confirmed by future studies on more male and female specimens. However the forewing color of P. nigricornis is stable on its entire distributional area and the depigmentation process is also stable in the perypatric speciation determining even the subgroup formations evolved by characteristic forewing colour and pattern.


Description. Description by Zetterstedt (1840:1065–1066): "tota testacea, pedibus pallidioribus, alis flavescentibus, subpubescentibus (major) ♂♀ (long corp. 4–4.5, al. exp. 11–12 lin.). "Hab. in Lapponia Suecica raro, sed ad littora maris glacialis Nordlandiae Finmarkiaeque Norvegicae, ex gr. ad Tromsoe et Alten, mense Jul. freq. (Lappon. – Scania, Sept. in copula). Mas et Fem. Inter majores. Tota testacea aut rufa, pedi-

Figures 7-9. Potamophylax testaceus (Zetterstedt, 1840) stat. nov. male. 7 = paramere in dorsal view, 8 = phallic organ in left lateral view, 9 = aedeagus in ventral view.

**Potamophylax elegantulus new species subgroup**

The narrow longitudinal pale light stripes present in the cells on the dark forewing of *Potamophylax nigricornis* have widened to light bands and the dark background disappeared and reduced to narrow stripes along the longitudinal veins. As a result the forewing was depigmented from light-striped dark background to dark-striped light background at all of the newly evolved species on the Balkan peninsula to Turkey. The complex pattern of the parameres is modified into simpler pectinate pattern, characterized by long or gradually apicad shortening and less arching spine-like setae. The ventral subapical heel on the aedeagus is reduced, extremely enlarged, or double layered. Doubled heels enclose some concavity.

**Potamophylax apados** Oláh & Chvojka sp. nov.  
(Figures 10–12)

**Diagnosis.** This light coloured species having dark striped forewing belongs to the *Potamophylax elegantulus* species subgroup, but differs from all the known species by the extremely elongated subapical ventral heels on the bifid head of the aedeagus as well as by the very short, abbreviated setae on the ventral side of parameres.

**Material examined.** Holotype. **Turkey**: Bolu Province, Abant Gölü Lake, brooklets, N40°36’00”, E31°17’00”, 13.VI.1998, leg. P. Chvojka (1 male, NMPC). Paratypes same as holotype (2 males NMPC, 2 males OPC, 2 male SCHU). Same as holotype, but 7.VII.1993 leg. O. Hovorka (1 male, NMPC)

**Figures 10-12.** *Potamophylax apados* sp. nov. male. 10 = paramere in dorsal view, 11 = phallic organ in left lateral view, 12 = aedeagus in ventral view.
Etymology. *apados* from “apadós” ebbing of in Hungarian refers to the very short abbreviated setae on the parameres.

Description. Male (in alcohol). Body and wing colour faded stramineous, possibly forewing stripe-patterned when alive. Periphallic organs are typical for the species group. Aedeagus almost parallel-sided in ventral view; bulging slightly below the ventral heels. Ventral heels extremely elongated spine-like. Apices parallel-sided, not tapering and not spatulate. Paramere shaft with mesad turning apical third accompanied by a single subapical seta closely adhering to the shaft. Nine short upward curving setae present on the ventrum of the shaft, almost invisible in dorsal view.

*Potamophylax elegantulus* (Klapálek, 1899) stat. nov.

(Figures 13–17)

Stenophylax nigricornis var. elegantulus Klapálek 1900:673.

Diagnosis. This species having dark striped forewing was described as a variant. The original description of *Potamophylax nigricornis var. elegantulus* is based upon the characteristic forewing pattern. Klapálek has not cleared the abdomen and thus he was unable to detect the significant modifications in the structure of the phallic organ. The ability of the aedeagal head to extend so much laterad during erection has been never observed at any specimens of *P.nigricornis*. Compared to *P.* nigricornis* the paramres has lost the basal quadratic basement of the shaft; sigmoid pattern reduced; the basal tuft of 5 strogly mesad curving setae shortened, straightened, doubled and spread up to the end of the shaft; apical seta fused to the end of the shaft, alveolus almost indiscernible. Female vaginal sclerite complex rounded, not elongated longitudinally, and has short sclerotized opening on the spermathecal process, not long; transversal bursal sclerate long, not short.

Figures 13-17. *Potamophylax elegantulus* (Klapálek 1899) stat. n. male. 13 = paramere in dorsal view, 14 = phallic organ in left lateral view, 15 = aedeagus in ventral view, 16 = expanded aedeagus in ventral view, 17 = dorsal profile of the vaginal sclerite complex.
Material examined. The original description in 1899 was based on 6 males and 2 females specimens without any type labels. Here we have designated lectotype and selected allotype. Lectotype. Bosnia-Herzegovina: Vrelo Bosna, 16 May (1 male, K369, NMPC). Allotype. same as lectotype (1 female: K373, NMPC). Paralectotypes. same as lectotype (1 male: K371, 1 male: K264, 1 male: K265, 1 male: K370, 1 male: K372; NMPC). Trebević leg. Winnegth (1 female, K374, NMPC).


Description. Male (pinned). Forewing pattern clearly dark striped on all the old pinned syntypes. Periphallic organs are typical for the species group. Aedeagus bulging below the ventral heels and the bifid head is highly produced laterad at half of the males. This laterad extended possibly erected state is never observed in any of the Potamophylax nigricornis populations. Subapical ventral sclerotized heels blunt triangular with varying pointed profile. End of lateral arms triangular, not tapering, and not spatulate. Paramere shaft slightly sigmoid; terminal seta fused, without alveolus; subapical seta almost as long as the terminal. 9-10 shortened, almost straight setae spread from base to subapical.

Female. We have cleared the abdomen of the two females in the syntype series and of the newly collected female. The dorsal profile of the vaginal sclerite complex of the three females is as short as wide; the sclerotized opening of the spermathecal duct on the spermathecal process is short and located on the middle of the sclerite; the transversal sclerite of the duct of bursa copulatrix is long.

Potamophylax fules Oláh & Ibrahimi sp. nov.

(Figures 18–20)

Diagnosis. This species having dark striped forewing belongs to the Potamophylax elegantulus species subgroup. Close to P. ureges sp. nov. but differs by having the ventral subapical double layered heel with lateral plates abbreviated; the lateral plate is longer at P. fules; as a result the mesal plate is long exposed free in lateral view; pectinate patterned setae distributed along to the subapical region, not limited to the basal half of the paramere shaft.

Description. Male (in alcohol). Body dark coloured, forewing dark striped. Forewing 19 mm. Periphallic organs are typical for the species group. Aedeagus almost parallel-sided in ventral view; ventral subapical heels double layered enclosing a concavity. Apices of bifid aedeagal head paralle-sided, not tapering. Paramere shaft straight with broad basement. regularly tapering apicad; setae almost equal long, pectinate patterned and distributed up to subapicad.

Etymology. fules from “füles” auriculate in Hungarian, refers to the ear-shaped small corner produced by the abbreviated lateral plate of the double-layered ventral subapical heel on the aedeagus.

Potamophylax hasas Oláh sp. nov. (Figures 21–24)


Diagnosis. Close to P. kethas sp. nov. but differs by having single belly on the aedeagus without a strong constriction midway, apical seta on paramere long, not short; basal setae four, not eight. Kumanski’s original male and female specimens were examined designated as allotype and paratype and compared to the holotype.


Description. Male (in alcohol). Body dark coloured, forewing with dark striped-pattern. Periphallic organs typical for the species group. Aedeagus with bulging lateral bellies in ventral view; ventral subapical heels double layered enclosing a concavity. Apices of bifid aedeagal head parallel-sided, not tapering. Paramere shaft straight with broadening basement and regularly tapering apicad; setae pectinate patterned; apical seta is the direct continuation of the shaft without alveolus; five basal setae curving mesad two setae located subapical.

Etymology. hasas from “hasas” bellied in Hungarian, refers to the subapical lateral bulging on the aedeagus.

Potamophylax kethas Oláh sp. nov. (Figures 25–27)

Diagnosis. Close to P. hasas sp. nov. but differs by having double bellies on the aedeagus with a strong constriction midway, apical seta on paramere short, not long; basal setae eight, not four.


Description. Male (in alcohol). Body dark coloured, forewing dark striped. Periphallic organs are typical for the species group. Aedeagus with pronounced constriction midway producing double bellies. Apices of bifid aedeagal head parallel-sided, slightly spatulate. Paramere shaft straight with very broad subquadratic basement and tapering apicad; single apical seta accompanied with a minute spine; basal tuft of eight setae followed by two subapical setae.
Figures 21-24. *Potamophylax hasas* sp. nov. male. 21 = paramere in dorsal view, 22 = phallic organ in left lateral view, 23 = aedeagus in ventral view, 24 = dorsal profile of the vaginal sclerite complex.

Figures 25-27. *Potamophylax kethas* sp. nov. male. 25 = paramere in dorsal view, 26 = phallic organ in left lateral view, 27 = aedeagus in ventral view.
Etymology. kethas from “két has” double bellies in Hungarian, refers to the strong constriction midway on the aedeagus resulting double bellies in ventral view.

**Potamophylax lemezes Oláh & Graf sp. nov.** (Figures 28–30)

**Diagnosis.** This species having no double layered heels (P. ureges, P. fules), no bellies on aedeagus (P. hasas, P. kethas), no elongated pointed heels (P. apados) and no broad paramere shaft (P. schmidt) has resemblance in the subgroup to P. elegantulus, but differs by having the unique lateral plate developed on the aedeagus subapicad, and by the differently patterned setal structure on the paramere shaft.


**Description.** Male (in alcohol). Body dark coloured, forewing dark striped. Periphalllic organs are typical for the species group. Aedeagus with pronounced elongated lateral plate subapicad; apices of bifid aedeagal head paralle-sided, slightly spatulate. Paramere shaft straight and tapering apicad; basal tuft of four-six setae and single subapical spine-like seta present.

**Female.** The dorsal profile of the vaginal sclerite complex almost regular diamond-shaped. Spermathecal process located above the middle line.

**Etymology.** lemezes from “lemezes” laminate in Hungarian, refers to lateral laminar plates present subapicad on the aedeagus.

![Figures 28-30](image)

**Potamophylax schmidt Marinković, 1971** (Figures 31–38)

*Potamophylax schmidt* Marinković 1971:80–83 (South-east Bosnia)

*Potamophylax schmidt* Marinković 1971:144 „South-east Bosnia, ♂♂ ♀♀ in many small brooks on the mountain Zelengora and Maglić.”

**Diagnosis.** Type specimens of this species were lost. We have collected only females and effort to borrow males failed. Male drawings are reproduced from the original description and drawings. Compared to other species of this group the reconstructed lateral view of phallic organ and the dorsal view of the paramere with setal formation are rather particular. We have examined the
available 10 females. Dorsal profile of the vaginal sclerite complex as well as the spermathecal process and the bursal sclerite are, as usual, very stable indicated by the narrow range of phenothpic variation.


**Potamophylax ureges Oláh sp. nov.**

(Figures 39–42)

**Diagnosis.** This beautiful species having dark striped forewing belongs to the Potamophylax elegansubalus species subgroup. Close to *P. fules* sp. nov. but differs by having the ventral subapical double layered heel with mesal and lateral plates almost equal enclosing a concavity; the lateral plate is shorter at *P. fules*, pectinate patterned setae distributed only on the basal half of the paramere shaft, not along to subapical region.


**Description.** Male (in alcohol). Body dark coloured, forewing dark striped. Periphallic organs are typical for the species group. Aedeagus almost parallel-sided in ventral view; ventral subapical heels double layered enclosing a concavity. Apices of bifid aedeagal head broad paralleled, not tapering. Paramere shaft straight with broad basement and regularly tapering apicad; setae almost equal long, pectinate patterned and restricted to the basal half.

**Etymology.** ureges from “üreges” supplied with hollow in Hungarian, refers to the concavity enclosed by the double-layered heels.

**Potamophylax horgos new species subgroup**

Depigmentation produced the forewing background paler and the narrow longitudinal light stripes present in the cells more obsolete. The subgroup has evolved along west and south peripheries of the Alps and in the direction of the Apennine peninsules. The complex pattern of the parameres modified into a simpler fan-like formation, characterized by strong, closely set mesad arching regular spine-like setae. The ventral subapical heel on the aedeagus modified into serrated, hooked, or spiny rim armed with various numbers of short peg-like prickles on the ventromesal surface.

**Potamophylax fureses Oláh, Lodovici & Valle sp. nov.**

(Figures 43–45)

**Diagnosis.** This species is most close to *P. tuskes* sp. nov. but differs by having lateral rim on the ventral subapical heels narrow, but present with serrated margin; apices of the bifid aedeagal head spatulate; setal fan of the paramere is shorter. The stabilities of these diverging traits must be examined on several newly collected specimens, including examination of the vaginal sclerite complex.

**Material examined.** Holotype. Italy: Toscana-Firenze, Marradi (FI), Monte Bruno, N44.0259 E11.6786, 700 m, 24.VIII.2003, leg. A. Usvellii (1 male, CNSMB). Paratype. Same as holotype (1 male, OPC).

**Description.** Male (in alcohol). Forewing paler, longitudinal stripes obsolete. Forewing length 19 mm. Periphallic organs are typical for the species group. Aedeagus constricted midway; apices of the bifid aedeagal head spatulate; ventral subapical heel produced into narrow serrated rounded rims. Paramere straight in dorsal, ventrad curving in lateral view; fanlike formation of short and strong spine-like setae is very short.

**Etymology.** fureses from “fürészes” serrated in Hungarian, refers to the serrated margin of the rim of the ventral subapical sclerotized heels on the aedeagus.
Figures 39-42. *Potamophylax ureges* sp. nov. male. 39 = paramere in dorsal view, 40 = phallic organ in left lateral view, 41 = aedeagus in ventral view, 42 = dorsal profile of the vaginal sclerite complex.

Figures 43-45. *Potamophylax fureses* sp. nov. male. 43 = paramere in dorsal view, 44 = phallic organ in left lateral view, 45 = aedeagus in ventral view.
Potamophylax horgos Coppa & Oláh sp. nov.  
(Figures 46–49)

Diagnosis. This species differs from the other species of the subgroup by having the longest fan on the paramere and hook formation, no flange or rim formation developed on the ventral subapical heel.


Description. Male (in alcohol). Forewing paler, longitudinal stripes obsolete. Forewing length 20 mm. Periphallic organs are typical for the species group. Aedeagus constricted midway; apices of the bifid aedeagal head laterad directed, varying between tapering and spatulate; ventral subapical heel produced into hook formation with few short teeth ventrad between the hooks. Paramere slightly sigmoid with fanlike formation of
short and strong spine-like setae; the fan is long from the tip almost to the basement. Female with rather regular diamond-shaped dorsal profile of the vaginal sclerite complex.

**Etymology.** *horgos* from “horgos” hooked in Hungarian, refers to the hook-like shape of the ventral subapical sclerotized heels on the aedeagus.

**Potamophylax peremes Oláh, Lodovici & Valle sp. nov.**

(Figures 50–52)

**Diagnosis.** This species is most close to *P. fureses* sp. nov. but differs by having lateral rim on the ventral subapical heels fully covered by short peg-like teeth; apices of the bifid aedeagal head narrowing, not spatulate; setal fan of the paramere is longer. The stabilities of these diverging traits must be examined on several newly collected specimens, includind examination of the vaginal sclerite complex.


**Description.** Male (in alcohol). Forewing paler, longitudinal stripes obsolete. Forewing length 19 mm. Periphallic organs are typical for the species group. Aedeagus constricted midway; apices of the bifid aedeagal head narrowing; ventral subapical heel produced into densely dentate rim. Paramere straight both in dorsal and lateral view; fanlike formation of short and strong spine-like setae is moderately short.

**Etymology.** *peremes* from “peremes” supplied with rim in Hungarian, refers to the rounded rim shape of the ventral subapical sclerotized heels on the aedeagus.

**Potamophylax tuskes Oláh, Lodovici & Valle sp. nov.**

(Figures 53–55)

**Diagnosis.** This species is most close to *P. fureses* sp. nov. but differs by having lateral rim on the ventral subapical heels fully covered by short peg-like teeth; apices of the bifid aedeagal head narrowing, not spatulate; setal fan of the paramere is longer. The stabilities of these diverging traits must be examined on several newly collected specimens, includind examination of the vaginal sclerite complex.

**Material examined.** Holotype. *Italy*: Mezzanego (GE), P.sso del Bocca, Parco Aveto T. L. Foresta Demaniale M.te Zatta, 1000 m, 31.VII.2009, leg. V. Raineri (1 male, CNSMB).

**Description.** Male (in alcohol). Forewing paler, longitudinal stripes obsolete. Forewing length 19 mm. Periphallic organs are typical for the species group. Aedeagus has broad rim subbasad; apices of the bifid aedeagal head narrowing apicad; ventral subapical heel produced into rounded rims. Paramere straight with bulbous basal region and developed fanlike formation of short and strong spine-like setae; the fan is long from the tip to midway.

**Etymology.** *tuskes* from “tüskés” dentate in Hungarian, refers to the short teeth covered rim of the ventral subapical sclerotized heels on the aedeagus.

**Potamophylax simas new species subgroup**

Similarly to *P. elegantulus* species subgroup the complex pattern of the ancestral parameres is modified into simpler pectinate pattern, characterized by long and gradually apicad shortening row of less arching spine-like setae. However the forewing is pale, not dark-striped on light background. The subgroup distributed in Massif Central, Pyrenees and an isolated taxon in the White Carpathians. Three species belong to this subgroup: *P. mista* Navas stat. nov., *P. sp. nov.* and *P. simas* sp. nov.

**Potamophylax sp.**

(Figures 56–58)

Figures 50-52. *Potamophylax peremes* sp. nov. male. 50 = paramere in dorsal view, 51 = phallic organ in left lateral view, 52 = aedeagus in ventral view.

Figures 53-55. *Potamophylax tuskes* sp. nov. male. 53 = paramere in dorsal view, 54 = phallic organ in left lateral view, 55 = aedeagus in ventral view.
Figures 56-58. *Potamophylax* sp. nov. male. 56 = paramere in dorsal view, 57 = phallic organ in left lateral view, 58 = aedeagus in ventral view.

*Diagnosis.* Forewing colour is ancestral gray. Most similar to *P. nigricornis*, but differs by having gonopods more expanded; ventral subapical heels rounded without tooth-shaped pointed plate; ancestral complex seatal pattern simplified. More male and female specimens are required to examine and to confirm the divergence of this taxon. It could be an atavistic species (Oláh in prep.).


*Description. Male (in alcohol).* Forewing colour gray, forewing length 17 mm. Periphalllic organs are typical for the species group except gonopods slightly expanded in oblique transversal plane. Aedeagus almost parallel-sided in ventral view; bulging slightly below the ventral heels. Ventral heels forming rounded plate without tooth-shaped pointed corner. Apices parallel-sided, not tapering and not spatulate. Paramere shaft straight. Number of spine-like setae seven: two apical, one subapical and four regularly set from subapical to the middle of the shaft.

*Potamophylax mista* (Navas, 1918) stat. nov.

(Figures 59–62)


*Diagnosis.* Close to *P. simas* sp. nov. but differs by having ventral subapical heel with small pointed tooth; setal pattern with basal tuft of setae curving mesad, the dorsal profile of the vaginal sclerite complex long, not short. All the specimens examined from the Pyrenees collected near to the locus typicus of both taxa described by Navas belong to this species.

*Material examined. France:* Departement Pyrenees-Atlantiques, Vieille Aure, Nest d’Oule, 1830 m, 10.VII.2009, leg. G. Coppa (2 males, 1 female, CPC; 2 males, 2 females, OPC). Departement Pyrenees-Atlantiques, Aragnouet, Neste de Couplan, 1380 m, 10.VII.2009, leg. G. Coppa (1 male, CPC). Departement Pyrenees-Atlantiques, Aragnouet, 1425 m, 8.VII.2009, leg. G. Coppa (2 males, 1 female; CPC). Departement Pyrenees-

Description. Description by Navas (1918:44–45): „Similar to var. testacea Zett. Et typo. Ala anterlor griseo-susca, pubescentia sat densa; striis digitiformibus longitudinalibus testaceis vel pallidisw in plerisque cellulis apicalibus.”

Remarks. Navas (1918) named his taxon as mista but he did not indicate the origin of the name and we treat mista as a noun in apposition.

**Potamophylax simas** Oláh & Coppa sp. nov.

(Figures 63–66)

Diagnosis. Most close to *P. mista* (Navas), but differs by having ventral subapical heel without pointed tooth; setal pattern with basal setae less curving mesad; the dorsal profile of the vaginal sclerite complex short, not long.


Description. Male (in alcohol). Forewing colour pale, forewing length 19 mm. Periphallic organs are typical for the species group. Aedeagus is parallel-sided in ventral view. Ventral heels forming rounded plate without tooth-shaped pointed corner. Apices of the aedeagal fifid head parallel-sided, not tapering, and not spatulate. Paramere shaft slightly sigmoid, its basement enlarged. Spine-like setal pattern pectinate, basal setae long, shortening subapicad, apical setae accompanied with a small seta.

Female. We cleared the abdomen of the ten females. The dorsal profile of the vaginal sclerite complex is stable, as short or shorter than wide; the sclerotized opening of the spermathecal duct on the spermathecal process is short and located on the middle of the sclerite; the transversal sclerite of the duct of bursa copulatrix is medium long.

Etymology. simas from “simá” plain or smooth in Hungarian refers to the simple parallel-sided aedeagus in ventral view and to the rounded ventral subapical heel without any pointed or tooth-like process.
Figures 59-62. *Potamophylax mista* (Navás, 1918) stat. nov. male. 59 = paramere in dorsal view, 60 = phallic organ in left lateral view, 61 = aedeagus in ventral view, 62 = dorsal profile of the vaginal sclerite complex.

Figures 63-66. *Potamophylax simas* sp. nov. male. 63 = paramere in dorsal view, 64 = phallic organ in left lateral view, 65 = aedeagus in ventral view, 66 = dorsal profile of the vaginal sclerite complex.
Acknowledgements – We sincerely appreciate the kind cooperation in supplying material, of Dr. Stoyan Beshkov, head of Invertebrates Department, National Museum of Natural History, Sofia and of Professor Marcos Gonzalez, Department of Zoology and Physical Anthropology, Faculty of Biology, University of Santiago de Compostela.

REFERENCES


Oláh et al.: The Potamophylax nigricornis group (Trichoptera, Limnephilidae)


OLÁH, J. & ITO, T. (2013): Synopsis of the Oxyethira flavicornis species group with new Japanese Oxy-


