A new species of *Hedyselmis* Hinton and notes on the phylogeny of the genus (Coleoptera: Elmidae)

FEDOR ČIAMPOR Jr.¹,³ & ZUZANA ČIAMPOROVÁ-ZAŤOVIČOVÁ²

Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84506, Bratislava, Slovakia.
E-mail: ¹f.ciampor@savba.sk, ²zuzana.zatovicova@savba.sk
³Corresponding author

Abstract

A new species, *Hedyselmis belatani* sp. nov., is described from West Malaysia, based on morphological characters. The description includes detailed drawings of the most important structures. Four fragments of DNA, three mitochondrial (cox1, cob, rrnL) and one nuclear (SSU), were sequenced and compared with *H. opis* Hinton and other elmid species. The taxonomic position of *H. belatani* sp. nov., its distribution and the phylogeny of the genus are discussed.

Key words: Coleoptera, Elmidae, *Hedyselmis*, new species, morphology, phylogeny, DNA, Malaysia

Introduction

Elmid research looks back at almost 180 years of history. This family includes more than 1300 species, but reliable information on their phylogeny is still lacking (Kodada & Jách 2005; Jách & Balke 2008). Molecular analyses have hardly been employed in Elmidae. Actually, there are only three papers on elmids dealing with DNA sequencing (Guglielmino & Olmi 2001; Čiampor Jr. & Ribera 2006; Čiamporová-Zaťovičová et al. 2007).

Filling this gap, we decided to start with smaller taxonomic groups, trying to reconstruct their relationships, subsequently including these data in the general picture of the entire family. In the present paper we are dealing with the genus *Hedyselmis* Hinton, which, although it includes only three species (including the species newly described here), is a most remarkable genus, which presents several morphologically unique characters (Hinton 1976; Jách & Boukal 1997). This paper is aimed at description of a new species of *Hedyselmis*, as well as at discussing its distribution and phylogeny, based on DNA sequence data.

Material, methods & abbreviations

Morphological analyses. The morphology of the new species was studied by using a Nikon SMZ-1B stereomicroscope with diffuse lighting at magnifications up to 140x; genitalia were examined as temporary slides using a Leica DM1000 transmitted light microscope at magnifications up to 600x. Drawings were made with a drawing device (Leica L 3/20). Metric characters were measured to the nearest 0.05 mm using a Nikon SMZ-1B with an ocular grid.
Molecular methods. The holotype was used for non-destructive DNA extraction. The extracted DNA is stored in the collection of the authors, ref. No. FZ-020. DNA was extracted from the whole specimen using the Qiagen DNeasy tissue kit. Four fragments were amplified using PCR; three mitochondrial (826 bp from the 3’ end of the cytochrome oxidase subunit I (cox1), 358 bp of the cytochrome b apoenzyme (cob), and 835 bp comprising the 3’ end of the rrnL (16S rRNA), the adjacent transfer RNA leucine 2 (tRNAleu), and part of NADH dehydrogenase subunit 1 (nad1)) and one nuclear (602 bp of the 5’ end of the ribosomal 18S rRNA gene, SSU) (for the primers used see Čiampor Jr. & Ribera 2006). Amplification products were purified using Qiagen Qiaquick PCR purification columns and sequenced in both directions. Sequences were sent to GenBank and have accession numbers: EU311731 (cox1), EU311732 (rrnL), EU311733 (SSU), EU311734 (cob).

Phylogenetic analyses. Protein-coding genes were not length variable and there was little variation in the ribosomal genes within Elmidae (length of the SSU fragment was between 626 and 628 bp; of the rrnL fragment between 817 and 827 bp). The sequences were aligned manually. The combined data matrix (all four genes) was analyzed using parsimony in PAUP* software version 4.0b10 (Swofford 2002), with a TBR heuristic search of 10,000 replicates and the option ‘save multiple trees’ activated. For comparison with the Bayesian probability results (see below), gaps in all searches were coded as missing characters, although treating them as a 5th character state did not change the topology of the trees (not shown). Node support was measured using Bremer Support (PBS) values (Bremer 1994) on constraint trees generated by means of TreeRot.v2 (Sorenson 1996) and non-parametric bootstrapping (Felsenstein 1985) using 1,000 pseudoreplicates of 50 random additions each.

Bayesian analyses were executed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), using a GTR + I + G model as selected by Modeltest 3.6 (Posada & Crandall 1998), with the parameters estimated for each partition (i.e. gene fragment). Searches were executed with default priors (uniform probabilities) starting with random trees with three heated and one cold Markov chains for 1,000,000 generations, sampled at intervals of 100 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time and the point when the likelihood values reached a stable equilibrium was visually determined. The parameter estimation (including tree topologies) obtained before reaching the stationary were discarded as a ‘burn-in’, and only the trees sampled after that point were considered (Huelsenbeck & Ronquist 2001).

Results

**Hedyselmis belatani** sp. nov.

Type locality: Malaysia, Kelantan, Hutan Lipur Lata Belatan, 5°38’35”N, 102°35’27”E (ca 35 m a.s.l.), 5 m wide stream, flowing slowly through primary lowland forest.

Material examined: Holotype ♂ Vienna Natural History Museum, Austria: “Malaysia, Kelantan, Kuala Krai env., stream in Hutan Lipur Lata Belatan, 5°38’35”N, 102°35’27”E, 7.6.2006, ca 35 m a.s.l. Čiampor & Čiamporová-Zaňovíčová lgt.”.

Differential diagnosis. Based on several morphological features, it is likely that *H. belatani* is more closely related to *H. gibbosus* Jäch & Boukal, from which it differs in: male protibia less regularly excavated; apex of last ventrite not excised; penis slightly longer; fibula with apex rounded; processes of phallobase thinner, more distinctly curved, lateral processes present, apices of admedian processes subtriangular with group of small spines. From *H. opis* Hinton it differs mainly in body shape, form of median pronotal groove, surface of ventral sclerites, shape of ventrite 5 and male genitalia.

Description. Habitus (Fig. 1). Body form obovate, length (pronotum + elytra) 4.1 mm, width 2.0 mm. Colour piceous except dark brown tibiae and slightly paler tarsi, antennae, mouth parts and lateral margin of elytra.
Head. Dorsal side densely micropunctured; genae and gula glabrous. Antennal segments 3–10 subequal, scape slightly curved, pedicel ca. 2/3 as long as scape, apical segment acuminate, with apex finely excised. Labrum wider than long, rugose, anterior margin setose; clypeus slightly longer than labrum, densely micropunctured; eyes moderately large, suboval in lateral view, convex in dorsal view, medially with raised, darkened margin.

Thorax. Pronotum almost as long as wide, widest behind middle, strongly convex; lateral margins explanate; anterior margin with two distinct admedian processes; anterior angles produced, rounded; median groove narrow, not reaching pronotal margins; prebasal admedian pits vestigial; surface densely micropunctured, except of almost glabrous prebasal portion. Prosternum densely plicate; sublateral ridges vestigial; prosternal process with margins micoreticulate, raised around coxae; posterior margin widely produced. Hypomera wide. Scutellum large, almost rounded, anterior margin straight; surface shiny with few tiny setae and indistinct lateral tubercles. Mesoventrite short and wide; disc with sinuate median carinae; margins around coxae

FIGURE 1. Habitus of Hedyselmis belatani sp. nov. (holotype).
 sharply raised. Metaventrite with lateral sides glabrous; disc longitudinally striate; longitudinal suture narrow, well impressed, reaching metaventral margins; admedian prebasal grooves arched around coxae; admedian prebasal tufts of setae present. In *Hedyselmis opis* and also in *Graphelmis* Delève, these tufts occur only in males. Thus it is suggested, that the prebasal setal tufts are secondary sexual structures characteristic for males. Elytra about twice as long as pronotum, parallel-sided in about anterior half, then converging toward rounded apices; lateral margins finely serrate, most distinctly explanate in apical third; strial punctures moderately deeply impressed; interval 1 flat, remaining intervals basally at least finely convex, intervals 3 and 4 fused in apical 0.25. Epipleura well developed. Legs glabrous, microreticulate; tibial cleaning fringes indistinct; protibia excavate subapically, with inner surface flattened; remaining tibiae simple, slightly widening towards apex; length of tarsomere 5 equal to combined length of tarsomeres 1–4; tarsi 1, 3, 4 with tufts of moderately long yellow setae; tarsal claws with well developed subbasal tooth.

Abdomen. Admedian keels of ventrite 1 reaching middle, not reaching posterior margin of ventrite; abdominal intercoxal process rounded; discs of ventrites 1–3 plicate, plication more developed on first two segments; ventrites 4–5 smooth; ventrites 2–3 mesally with small microreticulate array; posterior angle of ventrite 4 produced; apex of ventrite 5 rounded, admedially with setal tufts, lateral margin abruptly constricted.

Aedeagus (Figs 2–4). Penis elongate, corona present; fibula with apex rounded, setose; ventral sac well developed, densely setose. Parameres moderately reduced, dorsally situated, shorter than penis, with lateral processes, apices subtriangular with group of small spines on ventral side. Phallobase large, almost as long as penis, with apical „paramere-like“ processes; apical third of processes distinctly narrowed, apex strongly curved toward dorsal side of aedeagus.

Female unknown.

**Habitat.** The specimen was found on submerged wood in a slowly flowing lowland stream with sandy substrate and large boulders (Fig. 5).

**Distribution.** So far known only from the type locality (Fig. 6).

**Etymology.** Named after the type locality “Lata Belatan”.

**Phylogenetic analysis.** The combined sequence of *H. belatani* sp. nov. was included in the matrix used in Čiampor & Ribera (2006). The aligned matrix (cox1+cob+rrnL+SSU) had 2,717 characters. Within the whole dataset, there were 648 parsimony informative characters. Heuristic searches resulted in a single most parsimonious tree (consistency index CI=0.54, retention index RI=0.39) (Fig. 7). The sequence of *H. belatani* was grouped together with both sequences of the *H. opis* with high support (100% bootstrap, 100—posterior probability values x100 of the Bayesian analysis and 64—Bremer support). The clade *Hedyselmis*+*Graphelmis* was also well supported (99% bootstrap, 100—posterior probability values x100 of the Bayesian analysis and 23—Bremer support, Fig. 6).

In the Bayesian analyses the sampled ML values reached stationarity at ca. 50,000 generations, but the first 100,000 (i.e. 1,000 trees) were discarded as a burnin. The topology of the 50% majority rule consensus tree was almost identical to that of the parsimony tree, with the same highly supported nodes relating *Hedyselmis* species and the grouping of *Hedyselmis* with *Graphelmis*. The only difference was in grouping *Graphelmis obesa* Čiampor with *Hedyselmis* samples.

**Discussion**

Southeast Asia, due to its extremely high elmid species diversity represents a most important hotspot of the family (Jäch & Balke 2008). The diversity of the Malayan elmids is focused on Borneo, however there are still untouched areas in the continental part, where undescribed species occur. Despite extensive research and numerous descriptions of new species done so far (e.g. Kodada & Čiampor Jr. 2003; Čiampor Jr. 2004, 2006; Čiampor Jr. & Kodada 2004, 2006), with no doubt this region is inhabited by more species than expected.
Some genera, like *Graphelmis* or *Grouvellinus* Champion, are widely distributed across this region. However, there are many genera including only few species until now, which are found only locally, likely due to their special environmental requirements, e.g. *Loxostirus* Jäch & Kodada, *Rhopalonychus* Jäch & Kodada and *Graphosolus* Jäch & Kodada (Jäch & Kodada 1996a, b). The genus *Hedyselmis*, with its three species belongs to the latter group.

**FIGURES 2–4.** Aedeagus of *Hedyselmis belatani* sp. nov., 1) ventral view; 2) lateral view; 3) dorsal view. Scale=0.1mm.
FIGURE 5. Type locality of *Hedyselmis belatani* sp. nov., Malaysia, Kelantan, Kuala Krai env., stream in Hutan Lipur Lata Belatan.

FIGURE 6. Distribution of *Hedyselmis* species in West Malaysia: ■ *Hedyselmis opis*; ◆ *H. gibbosus*; ● *H. belatani* sp. nov. (Distribution of *H. opis* and *H. gibbosus* from Jäch & Boukal 1997).

Geographically the distribution area of *Hedyselmis* is distinctly fragmented across West Malaysia. The species occur in different basins. *H. opis* is known from more western parts, *H. gibbosus* was discovered at north-west and north of the Malay peninsula and the only known locality of *H. belatani* is found close to the east coast (Fig. 6). Due to the distribution of species localities and some morphological characters, it is sug-
gested, that the newly described *H. belatani* is more closely related to *H. gibbosus*. Unfortunately samples of the latter species usable for DNA analysis are not available, and thus we can not support this hypothesis by molecular data at present.

It was proposed recently, that *Hedyselmis* is closely related to *Graphelmis* (Jäch & Boukal 1997; Čiampor & Ribera 2006). Even though in the latter work paraphyly of *Graphelmis* in respect to *Hedyselmis* was suggested, the new *Hedyselmis* molecular data support validity of both genera. On the other hand, the new DNA data do not disprove their tight phylogenetic relationship, supported also by common morphological characters.

In the future it will be necessary to collect more material from Malaysia including samples of *H. gibbosus* preserved properly for DNA analyses and also to visit the neighbouring southern parts of Thailand, to increase our knowledge of the distribution of this genus.

**FIGURE 7.** Single most parsimonious tree obtained using the combined dataset. Above branches, posterior probability values (×100) of the Bayesian analyses (only if>0.5); below branches, bootstrap (only if more than 50%) / Bremer support values of the parsimony analyses.

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**References**


