# Austromesocypris bluffensis sp. n. (Crustacea, Ostracoda, Cypridoidea, Scottiinae) from subterranean aquatic habitats in Tasmania, with a key to world species of the subfamily 

Ivana Karanovic ${ }^{1, t}$, Stefan Eberhard ${ }^{2, f}$, Giulia Perina ${ }^{2.8}$<br>I Hanyang University, Department of Life Science, Seoul 133-791, Korea and Institute of Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Hobart, Tasmania 7001, Australia 2 Subterranean Ecology, Scientific Environmental Services, Suite 8, 37 Cedric Street, Stirling, Western Australia 6021, Australia<br>$\dagger$ urn:lsid:zoobank.org:author:3EF39C77-F792-44A1-B474-947D78F649B4<br>$\ddagger$ urn:lsid:zoobank.org:author:72BB5297-A591-4CC6-BAAF-C909512BD915<br>§ urn:lsid:zoobank.org:author:B141744C-1032-4550-9366-3264A5BCEAO1<br>Corresponding author: Ivana Karanovic (ivana.karanovic@utas.edu.au)

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#### Abstract

Austromesocypris bluffensis sp. n. is described and we report another species, Austromesocypris sp., both collected from subterranean aquatic habitats in Tasmania. This discovery adds a major taxonomic group to the already diverse invertebrate cave fauna of Tasmania, and is of interest because, globally, obligate subterranean aquatic species (stygobites) are poorly represented within the family Cyprididae. The genus Austromesocypris Martens, De Deckker \& Rossetti, 2004 is otherwise known to comprise entirely "terrestrial or semi-terrestrial" species. The second species is not described because only juvenile specimens were collected. Both species stand apart from their congeners by the carapace shape, which is rectangular in A. bluffensis and triangular and asymmetrical in the unnamed species. Another unique feature of the new species is the almost symmetrical uropodal rami. We also identify some broader systematic issues within the Scottiinae including the position of two New Zealand species, Scottia audax (Chapman, 1961) and S. insularis Chapman, 1963 in the genus, and point out their closer relationship to the Gondwana genera of Scottiinae, Austromesocypris and Mesocypris Daday, 1910, than to the Palearctic genus Scottia Brady \& Norman, 1889, based on the morphology of the maxillula and mandibula. The identity of the


Australian records of Scottia audax (Chapman, 1961), Austromesocypris australiensis (De Deckker, 1983) and the Boreal records of Scottia pseudobrowniana Kempf, 1971 are all considered doubtful. A key to the world species of Scottiinae is provided.

## Keywords

Ostracods, biodiversity, Cyprididae, Australia

## Introduction

Twelve freshwater podocopid ostracods belonging to the superfamily Cypridoidea and family Cyprididae are known from Tasmania (Table 1). The family Candonidae and representatives of the superfamily Cytheroidea, chiefly the family Limnocytheridae, have been recorded from Quaternary deposits (De Deckker 1982b) from Pulbeena and Mowbray swamps in north-west Tasmania. Darwinulidae are represented in Tasmania by one unnamed species of Penthesilenula Rossetti and Martens, 1998 (see Martens and Rossetti 2002) from the same Quaternary site.

Since their first appearance in the early Palaeozoic, podocopid ostracods have occurred in both marine and freshwater habitats, and today can be found from abyssal depths to small freshwater puddles, and even terrestrial environments (Pinto et al. 2003, 2004, 2005, 2008; Martens et al. 2004). All three major podocopid lineages, Cypridocopina, Darwinulocopina and Cytherocopina, have several representatives

Table I. List of ostracod species recorded from Tasmania. R - Recent, F - Fossil.

| Species | Record | Reference |
| :---: | :---: | :---: |
| Superfamily Cypridoidea |  |  |
| Australocypris robusta (De Deckker, 1974) | R | De Deckker (1977) |
| Candonocypris incosta De Deckker, 1982 | R | De Deckker (1982) |
| Diacypris spinosa De Deckker, 1981 | R | De Deckker (1981) |
| D. dietzi (Herbst, 1958) | R | Herbst (1958) |
| Kennethia cristata De Deckker, 1979 | R | De Deckker, 1979 |
| Mytilocypris praenuncia (Chapman, 1966) | R | Halse and McRae (2004) |
| M. splendida (Chapman, 1966) | R | De Deckker (1977) |
| M. tasmanica McKenzie, 1966 | R | McKenzie (1966), De Deckker (1977), Halse and McRae (2004) |
| M. mytiloides (Brady, 1886) | R | De Deckker (1977) |
| Mesocypris tasmaniensis De Deckker, 1983 | R | De Deckker (1983) |
| Newnhamia fenestrata King, 1855 | R | De Deckker (1978) |
| Sarscypridopsis aculeata (Costa, 1847) | R | De Deckker (1982a) |
| Candona tecta De Deckker, 1982 | F | De Deckker (1982b) |
| Candonopsis tenuis (Brady, 1886) | F | De Deckker (1982b) |
| Superfamily Cytheroidea |  |  |
| Limnocythere mowbrayensis Chapman, 1914 | F | De Deckker (1982b) |
| Gomphodella australiaca (Hussainy, 1969) | F | De Deckker (1982b) |

living in terrestrial habitats. Although Darwinulidae has no species restricted exclusively to terrestrial habitats, several species of the genera Vestalenula Rossetti and Martens, 1998 and Penthesilenula Rossetti and Martens, 1998 have been collected from terrestrial environments in Brazil (Pinto et al. 2003, 2004, 2005) (for the review of "semi-terrestrial ostracods" see Appendix).

Schornikov (1980) argued that ostracods found in terrestrial environments cannot be considered true terrestrial animals, as they were always covered by water, even if only a drop. This claim was rejected by Pinto et al. (2005) and Martens et al. (2004), who considered "terrestrial" all animals living outside free standing or flowing water. Danielopol and Vespremenau (1964) observed Scottia pseudobrowniana (Jones, 1850) move towards more humid areas, but closed its valves and stopped moving in non-saturated conditions, only becoming active again when in contact with water. Individuals of Scottia audax (Chapman, 1961) tended to move towards more damp parts of the litter as the available water decreased (Chapman 1961). Horne et al. (2004) recorded strong hydrotaxis in Terrestricythere elisabethae Horne, Smith, Whittaker and Murray, 2004, and death if desiccated for more than 10 minutes. The ability to inhabit environments such as leaf litter and other damp habitats may be advantageous, since it can allow a species to widen its area of distribution significantly and avoid predators occurring in fully aquatic habitats. It is easy to imagine how these tiny animals move, and one direct example is the finding of Microdarwinula zimmeri (Menzel, 1916) and S. pseudobrowniana on the floating fen soil (always in the part of the fen covered with water) in Romania (Danielopol and Vespremenau 1964). On the other hand, this way of life has its obvious limits, since such places are often subject to rapid desiccation. One more argument for not considering these ostracods truly adapted to the terrestrial environment is the fact that they are actually not widely distributed as would be expected with this adaptation. We know of only three exceptions where significantly broader distribution ranges, including trans-continental, are implied: Mesocypris pubescens Daday, 1910 has been recorded from Mount Kilimanjaro (Daday 1910), Kenya (Klie 1939) and South Africa (Lawrence 1953); S. audax from New Zealand (Chapman 1961) and widely throughout eastern Australia (De Deckker 1980, 1983); and S. pseudobrowniana from Europe, Asia (see Meisch 2000) and North America (Cole 1966, Külköylüoglu and Vinyard 2000). The broad distribution of these three species is discussed further in this paper and it is shown that they actually comprise additional unrecognized species. Redescriptions of these species is beyond the scope of this paper, but "species" as recognized herein are included in the key to species of Scottiinae presented at the end of this paper.

Animals living away from free-standing or flowing water have usually very hirsute shell and soft parts, which probably allows them to retain enough moisture in semi-terrestrial surroundings (Horne et al. 2004). Pinto et al. (2005) also consider parthenogenetic reproduction, commonly found in these ostracods, as one of the preadaptations to this environment. Although Chapman (1961) did not provide a detailed taxonomic description of $M$. audax, she gave a very interesting observation on the behaviour of these animals. She suspected that the juveniles hatch inside mother's shell,
since she observed large eggs within the shell with the outline of valves showing. Even more, in a live culture, in which young specimens were suddenly noticed, no laid eggs were seen. All this may suggest brooding care, never noticed before in Cypridoidea.

During the recent study of Tasmanian caves, two interesting new species were collected. One is named and described here, while the other is only briefly described but left as an open nomenclature, because only a juvenile specimen was collected. They both belong to the genus Austromesocypris Martens, De Deckker and Rossetti, 2004, a very peculiar and otherwise entirely "terrestrial" genus known only from Australia (Martens et al. 2004).

## Study area

Precipitous Bluff is located near the south coast of Tasmania in a remote and inaccessible part of the Tasmanian Wilderness World Heritage Area (Figure 1). The area remains essentially undisturbed by human activities except for occasional visits by bushwalkers and cavers. An extensive deposit of highly karstic and cavernous limestone of Ordovician age outcrops over about $10 \mathrm{~km}^{2}$ between $0-300$ meters above sea level (asl) on the western and southwestern flanks of Precipitous Bluff ( 1120 m asl) (Hughes 1957; Dixon and Sharples 1986) (Figure 2). The study area lies within the per-humid precipitation effectiveness province of Gentilli (1972). The nearest meteorological station is at Hastings located 25 km east-northeast, where mean annual rainfall is 1,416 mm . The cool, moist climate supports a dense vegetation cover of wet sclerophyll and cool temperate rainforest (Reid et al. 1999) (Figure 3).

The caves have developed by allogenic recharge from streams descending from the upper slopes of the mountain which sink underground on contact with the limestone. Autogenic recharge is also received from rainfall directly on the limestone outcrop, which percolates downward via a multitude of vertical shafts and fissures in the limestone (Figure 4). The descending subsurface flow paths coalesce at lower levels to form an integrated dendritic cave drainage network which discharges from resurgence caves situated at base level on the slope-plain juncture. Damper Cave is a major resurgence cave with more than one km of surveyed underground passages (Figure 5). The cave consists of a major conduit carrying a permanent flowing stream with a bedrock-gravelsandy bed, in addition to numerous small tributary streams and seepages.

The cave fauna at Precipitous Bluff was surveyed as part of a state-wide biodiversity survey of Tasmanian karsts undertaken by Eberhard et al. (1991). This study discovered that the Precipitous Bluff karst supported the richest assemblage of locally endemic and obligate cave-dwelling species in Tasmania (Eberhard 1996). Initially, most of this assemblage comprised terrestrial cave obligate species (troglobites) including described species of Coleoptera (Eberhard and Giachino 2011; Moore 1978), Diplopoda (Mesibov 2005), Opiliones (Hunt 1990; Hunt and Hickman 1993), synotaxid (Forster et al. 1990) and micropholcommatid (Rix and Harvey 2010) spiders, in addition to other undescribed species of Opiliones, Araneae,


Figure I. Tasmania, Australia, showing location of Precipitous Bluff study area.

Pseudoscorpionida, Oniscidea, and Collembola (Eberhard et al. 1991). A significant fauna of obligate aquatic subterranean species (stygobites) in the Precipitous Bluff caves was also indicated by the discovery of Australia's first stygobitic gastropod Pseudotricula eberhardi Ponder 1992, followed by the description of an additional seven species of cave dwelling hydrobiid gastropods (Ponder et al. 2005). Besides the hydrobiids, the sampled aquatic macrofauna included mayflies (Ephemeroptera) and crustaceans (Amphipoda and Syncarida), the latter possessing clear stygomorphies (loss of eyes, pigment and elongation of appendages) indicating their status as stygobites. The initial survey by Eberhard et al. (1991) focused on collecting macroinvertebrates, but Ponder et al. (2005) concluded that additional sampling, especially targeted towards aquatic micro- and meiofauna, was likely to further increase the richness of subterranean species known from the Precipitous Bluff karst. This possibility provided the impetus for the current study.

Figure 2. Precipitous Bluff ( 1140 m asl) showing heavily forested lower slopes which contain the karst and caves.


Figure 3. Typical cool temperate rainforest overlying the karst and caves.


Figure 4. Typical karst terrain and cool temperate rainforest vegetation in the study area.


Figure 5. Damper Cave entrance and stream resurgence. Collection sites were located approximately 20 to 80 m inside the cave entrance, see next figures.

## Field methods

Aquatic micro- and meiofauna were sampled from a variety of habitats in Damper Cave in May 2011 using $150 \mu \mathrm{~m}$ mesh plankton nets. Three types of aquatic habitats were sampled: (1) main stream (Site A); (2) small tributary stream (Site B); and (3) seepage water from dripping stalactites (Sites, C, D, E). To sample the main stream benthos, shallow interstitial and stream drift fauna, two 300 mm diameter plankton nets were installed in the main flow of the stream approximately 30 m upstream from the resurgence entrance (Figure 6). Benthic and shallow interstitial fauna was sampled by 'kick' sampling which involved walking upstream from the nets for a distance of approximately 50 m while 'kicking' the gravel-sandy sediments to dislodge fauna which was swept downstream into the nets. A small tributary stream approximately 20 m inside the resurgence entrance was sampled by placing a bucket and net underneath a vertical trickle (Figure 7). Seepage water from dripping stalactites was sampled in a similar manner. The nets were left in the cave for two days and then recovered, where after the net contents were elutriated and preserved in $100 \%$ ethanol.


Figure 6. Damper Cave main stream collection Site A with drift nets.

## Taxonomic methods

Specimens were dissected and mounted on microscope glass slides in Faure's medium, which was prepared following the procedure of Stock and von Vaupel Klein (1996). The dissected appendages were then covered with a coverslip and observed under the Leica L2 stereomicroscope and Leica DM 2500 compound microscope with N-plan objectives, and the drawings were made using a drawing tube. The photographs of ostracod shells were taken with a Leica M205 C microscope fitted with a Leica DFC420 digital camera, and montaged using the Leica Application Suite V3 software. All material is deposited at the Tasmanian Museum and Art Gallery (TMAG), Hobart.

Terminology. In the present paper the terminology for the most posterior appendage on the body, the so-called uropodal ramus, follows Meisch (2007). Terminology of other soft parts follows Broodbakker and Danielopol (1982), Martens (1987), and Meisch (1996).

Abbreviations used in text and figures. A1-antennula; A2-antenna; LV-left valve; L5-L7-fifth-seventh limb; Md-mandibula; Mxl-maxillula; RV-right valve; URuropodal ramus.


Figure 7. Damper Cave tributary stream collection Site B with net.

## Taxonomy

Class Ostracoda Latreille, 1806
Subclass Podocopa Müller, 1894
Order Podocopida Sars, 1866
Superfamily Cypridoidea Baird, 1845
Family Cyprididae Baird, 1845
Subfamily Scottiinae Bronstein, 1947
Genus Austromesocypris Martens, De Deckker and Rossetti, 2004
http://species-id.net/wiki/Austromesocypris
Type species. A. berentsae Martens, De Deckker and Rossetti, 2004; original designation.
Other species. A. australiensis (De Deckker, 1983); A. bluffensis sp. n.; A. tasmaniensis (De Deckker, 1983)

## Austromesocypris bluffensis sp. n.

urn:lsid:zoobank.org:act:A2DDB15B-AC3D-433C-9FFC-561AFBFDEAAD
http://species-id.net/wiki/Austromesocypris_bluffensis
Figures 8A, B, 9-11

Material examined. Holotype, female (TMAG 6206) Damper Cave, $43^{\circ} 30^{\prime}$ S, $146^{\circ} 35^{\prime}$ E, Precipitous Bluff, 90 km SW of Hobart, Tasmania, Australia, Site A, main stream, 30 m from entrance, 14 May 2011 (dissected on one slide), collected by R. and S. Eberhard, G. Perina, S. Catomore.

Diagnosis. Ostracods with smooth, transparent shell densely covered with setulae. Dorsal margin straight, anterior and posterior margins almost equally wide. Calcified inner lamella narrow. A1 with fused third and fourth segments. A2 with only two short swimming setae and two $t$-setae. Md-palp with pappose $\gamma$-seta. L5 with one a-seta and one d-seta. L6 with d2-seta, short e-seta, second and third endopodal segments fused, and long terminal claw. UR with row of long, strong setulae along posterior margin; both distal claws strong with strong spines; anterior seta long. Rami almost symmetrical, only one ramus with slightly shorter setulae along posterior margin. Genital field with one clear thumb-like projection.

Etymology. The species is named after its type locality.
Description of female. Carapace (Figs 8A, B; 9A). Rectangular in lateral view; 0.58 mm in length. Greatest height situated around middle length, equalling 0.26 mm , or $45 \%$ of length. Valves clearly asymmetrical, RV being shorter than left one. Dorsal margin straight on almost entire length, rounding towards posterior end and inclined with small recess towards anterior end. Both anterior and posterior ends rounded, anterior end more so and slightly wider than posterior one. Ventral margin straight or very slightly concave. Anterior and posterior inner calcified lamellae narrow. Marginal


Figure 8. A, B Austromesocypris bluffensis (Holotype) C Austromesocypris sp. A shell, view from the right side $\mathbf{B}, \mathbf{C}$ shell, view from the left side.



Figure 10. Austromesocypris bluffensis (Holotype): A A2 B L5 C Md-palp. Scales $=0.1 \mathrm{~mm}$.


Figure II. Austromesocypris bluffensis (Holotype): A UR, arrow indicating the genital process $\mathbf{B}$ attachment of the UR C L6 D forehead and upper lip $\mathbf{E L 7} \mathbf{F}$ detail of the distal end of L7. Scales $=0.1 \mathrm{~mm}$.
pore canals short, except in ventral region where enlarged. Surface densely covered with relatively short hair-like setae.

A1 (Fig. 9B, C). First segment hirsute, anteriorly with only one pappose seta, reaching middle length of following segment; posteriorly with two, almost equally long, pappose setae originating close to each other and near distal margin of segment. Wouters organ not observed. Following segment with very short Rome organ posteriorly and one pappose seta anteriorly, reaching middle of following segment. Above this seta cluster of setulae present. Third segment compound, representing fused segments three and four; point of their fusion clearly marked by one short pappose seta, which almost reaches distal margin of following free segment. Distal margin of fused segment with one posterior, short and smooth seta and one long, also smooth seta anteriorly. This seta as long as length of all segments combined. Segment following fused segments (fifth segment) very short with two long and smooth setae anteriorly and one shorter seta posteriorly. Penultimate segment with total of four anterior setae, all situated very close to each other, three being longer than length of all segments combined and one equalling $1 / 3$ of their length. Terminal segment with one long and two shorter setae, this segment with long aesthetasc y1 being eight times longer than terminal segment. Length ratios of last four segments equalling $1.8: 1: 1.5: 1$.

A2 (Fig. 10A). Coxa with three pappose, equally long setae: one situated more proximally (externally) and two more distally (internally) on segment. Coxa with three rows of small setulae. Basis laterally with cluster of setulae and one anterior seta, which reaches distal end of first endopodal segment. Exopod representing small plate with three setae: most anterior one distally pappose and reaching distal margin of first endopodal segment; middle one also pappose and much shorter; most posterior one even shorter and also smooth. Endopod 3-segmented. First segment with two strong setulae along anterior margin and row of short setulae antero-distally; aesthetasc Y situated in middle of posterior margin and reaching distal end of segment; postero-distal seta pappose and extends beyond middle of second endopodal segment. Two short swimming setae situated antero-distally on segment and only one reaches $1 / 3$ of second endopodal segment. Second endopodal segment with cluster of setulae mid-laterally; seta on middle of anterior margin distally pappose and reaches distal end of segment. Second endopodal segment posteriorly with two short aesthetascs: y1 situated more proximally and $y 2$ at distal margin. Same segment postero-medially with three $t$-setae: t 1 distally pappose and shorter than t 2 and t 3 . Second endopodal segment with three smooth and equally long $z$-setae on distal margin, all reaching middle of terminal claws. G2 claw equalling $2 / 3$ of G1 claw. G1 and G3 equally long and only slightly shorter than first endopodal segment. Terminal segment short with GM claw equalling $70 \%$ of first endopodal segment; Gm claw being $2 / 3$ of GM. Same segment with one additional thin seta which is as long as Gm claw; aesthetasc y3 of same length and proximally fused with one thin seta.

Forehead and lips (Fig. 11D). Hirsute with numerous sclerotized rods and rake like organ carrying about six blunt teeth.

Md (Fig. 10C). Exopod short carrying five plumose, vibratory setae. Palp 4-segmented. First segment posteriorly with three setae, one smooth and other two with one row of long and strong setulae; one of these setae bent. Alpha seta, usually present on this segment, not observed. Second segment anteriorly with three equally long pappose setae not reaching distal margin of following segment; posteriorly with three long, smooth setae, one shorter seta with setulae along one margin, and one short and plumed seta ( $\beta$-seta). Penultimate segment anteriorly with one very short seta and two long and smooth setae each exceeding distal end of terminal segment; posteriorly with total of four setae: two long and two shorter, one of which half as long as long setae, other $1 / 4$ length of these setae; $\gamma$-seta plumed, exceeding distal end of terminal segment. Terminal segment elongated ( 2.5 times longer than wide) with four distally curved claws and one seta half as long as claws.
$M x l$ (Figure 9D). Palp 2-segmented. First segment with three pappose and two smooth setae, all situated close to outer margin. Terminal segment 1.8 times longer than wide, with two claws (one fused with segment) and four setae. First endite distally with two long, pappose setae and five short claws; proximally with two short and smooth setae. Second endite with four short claws. Third endite with four claws (one fused with segment) and cluster of short setulae on anterior margin.

L5 (Fig. 10B). Exopod with two plumose vibratory setae. Endopod with three distal pappose setae, two being twice as long as third. Protopod with one pappose a-seta, one pappose d-seta and six distal pappose setae.

L6 (Fig. 11C). Basal segment (basis) with short pappose d2-seta and marginal rows of setulae. First endopodal segment with long anterior setulae, and three medial rows of shorter setulae; same segment antero-distally with short and pappose e-seta. Following segment compound, representing fused second and third segments, with f-seta near middle of anterior margin and distally with two setae (one being g-seta) and row of marginal setulae. Terminal segment with two thin setae (h1 and h3) and one strong claw (h2); latter gently serrated and 0.72 times as long as endopodal segments combined.
$L 7$ (Fig. 11E, F). Composed of three segments plus terminal pincer. First segment with three long, pappose setae ( $\mathrm{d} 1, \mathrm{~d} 2$ and dp ). Second segment with pappose e-seta reaching half length of following segment. Third segment compound, representing fused endopodal segments two and three, with f -seta near middle of anterior margin; g-seta absent. Terminal segment reduced and transformed into pincer organ. Seta h1 very thin and curved, h2-seta claw-like and distally pappose, seta h3 normal and almost as long as penultimate segment.
$U R$ (Fig. 11A). Posterior margin with groups of long and strong setulae; posterior seta situated close to distal margin, being pappose and more than half as long as posterior claw. Posterior claw only slightly shorter than anterior one; both claws strongly serrated. Anterior seta distally pappose and as long as anterior claw. Rows of setulae on posterior margin of one ramus slightly weaker. Length ratios between anterior margin of ramus and anterior and posterior claws equalling $2.9: 1.1: 1$.

Attachment of $U R$ (Fig. 11B). Distally bifurcate, with no additional ventral or dorsal branches.

Genital field (Figure 11A) with clear thumb-like projection (indicated by arrow on Figure 11A).

Males. Not known.
Remarks. Austromesocypris bluffensis sp. n. stands apart from all other species of the genus in having a completely flat dorsal margin of the carapace and almost symmetrical UR. In addition, it differs from the New South Wales species, $A$. berentsae Martens, De Deckker and Rossetti, 2004, in having only one segment on the A1 fused. Austromesocypris berentsae is the only species in the genus that has two segments fused $(3+4,5+6)$. Austromesocypris bluffensis differs from A. australiensis (De Deckker, 1983) by having structurally similar posterior setae on both UR. Only $A$. australiensis, among all species of the genus, has one seta transformed into a strong claw, the other being seta-like. The one previously described species from Tasmania, A. tasmaniensis (De Deckker, 1983), has much stronger spines along the posterior margin of the UR. The UR of the new species is the most similar to $A$. berentsae, but the former species has shorter spine-like setae along the posterior margin of the ramus. It appears that the gamma-seta on the Md-palp in $A$. berentsae is not pappose (see Martens et al. 2004, Fig. 3), while it is in the other three species. There are other differences in the chaetotaxy of the Md-palp between the new species and the other three, namely $A$. bluffensis has four setae postero-distally on the penultimate segment, $A$. berentsae has two, while the two species described by De Deckker (1983) have three. Moreover, there are only two setae antero-distally on the same segment in $A$. bluffensis, while all other species have three. It is interesting to note that the S2 seta on the first segment of the Md-palp is turned upwards only in $A$. bluffensis. We are uncertain if this character has any taxonomic significance, or whether this seta was twisted during slide preparation. However representatives of many Cyprididae subfamilies (Cypridopsinae, Cyprinotinae, Herpetocypridinae) and some Candoninae and Cyclocypridinae have the seta S2 similar to that of $A$. bluffensis (I. Karanovic personal observation).

## Austromesocypris sp.

Figures 8C, 12
Material examined. One juvenile female (A-7 or A-8) (TMAG 6207), Damper Cave, Precipitous Bluff, $43^{\circ} 30^{\prime} \mathrm{S}, 146^{\circ} 35^{\prime} \mathrm{E}, 90 \mathrm{~km}$ SW of Hobart, Tasmania, Australia, Tributary stream 20 m inside entrance, 14 May 2011 (dissected on one slide), collected by R. and S. Eberhard, G. Perina, S. Catomore

Descriptive notes. Triangular shell (Figs 8C, 12A) with left valve overlapping right one. Anterior and posterior margins rounded with long marginal hair-like setae. Surface not highly hirsute. Length 0.58 mm . UR (Fig. 12B) with anterior margin sparsely covered with strong setulae (same on both rami). Posterior seta pappose and only $1 / 3$ length of posterior claws. Both claws strongly serrated; posterior one only slightly shorter. Anterior seta pappose and longer than anterior claw.

Remarks. This species is assigned to the genus Austromesocypris Martens, De Deckker \& Rossetti, 2004 based on the appearance of the UR. The undescribed female


Figure 12. Austromesocypris sp.: A shell, external view from the right side B UR. Scales $=0.1 \mathrm{~mm}$.
was probably a late juvenile (A-7 or A-8), as indicated by the well-developed appendages (including the 6-segmented A1). However, the setae on the L6 and L7 had a swollen base and the oviducts, as well as the entire genital field, were undeveloped. Until now, no Scottiinae were known with such a triangular and asymmetrical carapace shape. An unnamed fossil species, Mesocypris sp., from the Pulbeena Swamp in Tasmania (De Deckker 1982) also has a highly arched carapace, but the posterior margin in this species is broader and the LV is not higher than the RV.

Associated fauna. Other taxa collected in the Damper Cave samples included: a) Copepoda (Diacyclops cryonastes Morton, 1985), Amphipoda (Paramelitidae), Isopoda (Styloniscidae), Oligochaeta (Phreodrilidae), Gastropoda (Hydrobiidae), Nematoda, Turbellaria (Tricladida), Diptera, Plecoptera, and Ephemeroptera from the main stream Site; and b) Isopoda (Janiridae: Heterias sp.) from the stalactite drip Site E.

## Discussion

## Ecology

No Ostracoda have previously been described from Tasmanian caves or other groundwater environments. Illife (1988) reported a collection of ostracods from Honeycomb Cave at Mole Creek in northern Tasmania; however these remain unidentified. As already noted, biospeleological collections to date have targeted macro-invertebrates while
aquatic micro- and meio-fauna have been poorly investigated (Ponder et al. 2005). The absence of Candoninae in Tasmanian caves may be an artefact of poor sampling effort, but it is also possible that this group is substituted here by Austromesocypris Martens, De Deckker \& Rossetti, 2004 species that have occupied a new habitat through the lack of competition with Candoninae, the dominant subterranean ostracod group elsewhere in the world (Karanovic 2007). Despite a substantial sampling effort during this study, only one complete specimen of each species has been collected. Subterranean ostracods are often rare in subterranean samples (Karanovic 2007, Karanovic and McKay 2010).

Discovery of cave dwelling ostracods described in this paper is of interest for two reasons. Firstly, members of Cyprididae are rarely found in subterranean waters. Secondly, the genus Austromesocypris is otherwise known to comprise entirely "terrestrial and semi-terrestrial" species. The rare finds of Cyprididae in subterranean waters are mostly records of morphologically unmodified taxa which occur as facultative or incidental inhabitants of groundwaters. The only documented case of an obligate association of cyprids with groundwaters involves the genus Pseudocypridopsis Karanovic, 1999, with two described stygobitic species from the Balkan Peninsula (Karanovic 1999, 2000). In Western Australia, where a great deal of sampling in wells and water bores has revealed a globally significant richness of stygobitic Crustacea (Eberhard et al. 2005, 2009), no truly stygobitic Cyprididae occur, although unmodified epigean species are not infrequently collected especially in wells which are open to the surface and therefore easily colonized by epigean species.

The collection of a whole specimen of $A$. bluffensis sp . n . in the main stream implies that this animal could have been living in the benthos or interstitial of the main stream, but also does not preclude an origin from a tributary stream or seepage waters, of which the latter two ultimately discharge into the main stream. The collection of valves in seepage water from dripping stalactites and a complete specimen of Austromesocypris sp. in the small tributary stream confirms the occurrence of Austromesocypris in these habitats also. Notwithstanding they might still have originated from the surface which lies only a few tens of meters above Damper Cave at the point where the collections were made. Certainly the cool temperate rainforest represents a near-permanently moist habitat for hygrophilous invertebrates including, potentially, terrestrial/semiterrestrial ostracods. The highly permeable karst at Precipitous Bluff ensures a close eco-hydrological connection between epigean and hypogean environments, confirmed by the occurrence of other normally epigean-terrestrial invertebrates such as charopid gastropods accidentally washed into the caves by percolating seepage waters (S. Eberhard personal observation).

## Adaptive morphology

Some adaptive morphologies, typically comprising numerous reductions, displayed in semi-terrestrial epigean ostracods are similar to those seen in aquatic subterranean ostracods as already pointed out by several authors (Danielopol 1978, Danielopol and

Betsch 1980, Pinto et al. 2005, 2008). Danielopol and Betsch (1980) also point out the close phylogenetic relationships between the semi-terrestrial Candoninae from Madagascar and their interstitial aquatic relatives. This may additionally support the view of Karanovic (2007) that the subterranean Candoninae genus Nannocandona Eckman, 1914 should belong to Terrestricandonini instead of Candonini because of the many morphological characters shared with members of the former tribe. It is also worth noting that the subfamily Timiriaseviinae not only contains the only freshwater semi-terrestrial cytheroid ostracod Intrepidocythere Pinto, Rocha and Martens, 2008 but also is the only cytheroid subfamily with true stygobiont species such as those in the genera Kovalevskiella Klein, 1963 and Gomphodella De Deckker, 1981.

The unusual shape of the two new Tasmanian species, one with trapezoidal shape, the other with triangular shape, suggests that these might also be true stygobites as such modification is very common among subterranean Candoninae (Danielopol 1978, Karanovic 2007). In addition to this potentially convergent stygomorphic character, typical for Ostracoda, the Austromesocypris from Precipitous Bluff also exhibit classic convergent regressive stygomorphic characters seen in subterranean Crustacea, including depigmentation, loss of eyes and reduction in body size. On the other hand, these Tasmanian specimens do not exhibit any other obvious stygomorphic characters such as elongation of appendages as seen in Candoninae and most other stygomorphic Crustacea. It should be noted that the dominantly terrestrial/semi-terrestrial group Scottiinae typically exhibit reduction in body size and size of segments, a gracile appearance, and anopthalmia.

## Systematics

Austromesocypris is according to Martens et al. (2004) most closely related to Mesocypris Daday, 1910 and they share a reduced number of free segments on the A1, fused second and third endopodal segments on the L6, a short h3 seta on the terminal segment of the same appendage, and asymmetrical caudal rami. Mesocypris is a paraphyletic taxon according to the cladistic tree obtained by Martens et al. (2004), and indeed many species deviate from the characters that should define the tribe Mesocypridini to which the two genera belong according to Martens et al. (2004). For example, at least two species have the second and third endopodal segments partly separated on the L6: M. terrestris Harding, 1953 and M. pauliani Danielopol \& Betsch, 1980. The h3 seta is longer in all Mesocypris species than in Austromesocypris and its length is somewhere in between for the latter genus and Scottia Brady \& Norman, 1889. Martens et al. (2004) stated in their revised diagnosis that in tribe Mesocypridini h3 is short, while in Scottiini it is claw-like. One of the Madagascar species, M. pauliani Danielopol \& Betsch, 1980 actually has a claw-like h3 seta. Another doubtful character of the tribe Mesocypridini is the morphology of the UR. While the posterior margins of the right and left ramus are indeed asymmetrical (one hirsute, other with teeth or spines) in all previously known species, only Austromesocypris australiensis has
one posterior seta transformed into a strong claw and the other seta-like. In all other Mesocypridini species this difference is much less obvious, especially in other species of the genus Austromesocypris.

All species of Austromesocypris have only two swimming setae on the A2, while in Mesocypris the number varies from six to two. There are, however, two characters which indicate the two genera are more closely related to each other than either is to the genus Scottia. Namely, the terminal segment on the Md-palp is much more elongate and the number of setae on the first segment of the Mxl-palp is reduced in Mesocypris and Austromesocypris in comparison to Scottia. According to Chapman's (1961) illustration of the Md of S. audax (Chapman, 1961) and Chapman's (1963) drawings of the Mxl of S. insularis Chapman, 1963, the two New Zealand species appear to be more closely related to members of Mesocypridini. Scottia audax was originally described in the genus Mesocypris, but De Deckker (1980) transferred it to the genus Scottia because of the morphology of the L6, namely the presence of the long, claw-like h3 seta, and at the same time also suggested that S. insularis Chapman, 1963 might belong to Mesocypris due to the short h3 seta. Both New Zealand species were retained in the genus Scottia by Martens et al. (2004), but those authors pointed out the unusual distribution of the genus, with two species, Scottia pseudobrowniana Kempf, 1971 widely distributed in the Holarctic (Meisch 2000) and S. birigida Smith, Matzke-Karasz, Kamiya \& Ikeda, 2002 endemic to Japan (Smith et al. 2002).

The position of the two New Zealand species is not certain at the moment, but they definitely should be excluded from the genus Scottia. While they share many characters with Mesocypris, most of which are variable, they also have a reduced number of segments on the A1 like Austromesocypris. Fusion of A1 segments can also be partial, as noted by Matzke-Karasz (1995) for S. audax. Namely, borders between segments $3+4$ and $5+6$ are incompletely formed and only visible on the interior side, a fact noticed by Martens et al. (2004) as well. Another character that may support a closer relationship of $S$. audax to Mesocypridini is the reduced number of rays on the exopod of L5. Namely, both Palearctic Scottia species have six rays, while the New Zealand species has only four (see drawings of Matzke-Karasz 1995). The number is further reduced to two or three setae in Mesocypris and Austromesocypris. This character needs to be taken with caution, because the exopod of L5 may be easily damaged during slide preparation and the setae easily fall off.

## Misidentified species

In our opinion Australian records of Scottia audax (Chapman, 1961) by De Deckker (1980) are incorrect, that material representing a different, as yet undescribed species. This is even more apparent from the drawings of $S$. audax provided by Matzke-Karasz (1995) who studied De Deckker's (1980) material. The most significant difference between the Australian species and S. audax is the morphology of the UR. The posterior seta is longer, stronger and more closely situated to the distal end of the ramus in the

Australian species, but the claws are stronger and the posterior margin of the ramus is covered with small teeth (or at least thick spines) in S. audax. Another finer detail is the appearance of the exopodal setae of the A2, which in the Australian species has one long and two short, subequal setae whereas in S. audax the two shorter setae are unequal in length, with one being at least twice as long as the other (as in A. bluffensis). The chaetotaxy of the Md-palp is also somewhat different, as all the dorsal setae in $S$. audax are longer than those of the Australian species. It is interesting that $S$. audax and the Australian species have the S 2 seta turned the same way as in $A$. bluffensis sp. n. and that all three species also have four setae postero-distally on the penultimate segment.

In our opinion De Deckker's (1983) report of Austromesocypris australiensis also represents two species which differ most significantly in the appearance of the hemipenis, prehensile palps and the number of the rosettes on the Zenker organ. One was found in far north Queensland, and the other in New South Wales and Victoria, but both were identified as $A$. australiensis by De Deckker (1983). Previous collections of $A$. tasmaniensis may also contain two species, as De Deckker (1983) observed some differences in the length of the shell and morphology of the UR between specimens taken from two localities. These samples need to be studied in more detail to draw an accurate conclusion.

Misidentified Scottiinae are not confined to Australian records. Reports of the "widely distributed" Scottia pseudobrowniana from Tennessee (Cole 1966) and Nevada (Külköylüoglu and Vinyard 2000) are also probably incorrect. The specimens from Tennessee (east) resemble S. pseudobrowniana more than those from Nevada (west). While the Nevada material has an almost straight posterior margin of the shell, the one from Tennessee has a rounded posterior margin, similar to that of S. pseudobrowniana, but with both ends being narrower than in the latter species. Specimens of the North American S. pseudobrowniana have shorter swimming setae on the A2 than the European S. pseudobrowniana. Indeed the setae in the Nevada material are so short that they only slightly overpass the first endopodal segment. Specimens of S. pseudobrowniana from the three separate geographic locations differ mostly in the morphology of the hemipenis. Although neither Cole (1966) nor Külköylüoglu and Vinyard (2000) labelled or drew this organ in detail, the outline they provide indicates enough differences to claim that three separate species are represented. Scottia pseudobrowniana has all segments on the A1 free, while Külköylüoglu and Vinyard (2000) illustrated a 6 -segmented A1. If this is correct, it would represent an outstanding character. It must be noted, however, that the illustrations of Külköylüoglu and Vinyard (2000) show limited detail and may not correctly show the relevant characters.

Scottia pseudobrowniana from Russia, as illustrated by Bronstein (1947), is very similar to the figures of the Nevada species of Külköylüoglu and Vinyard (2000), but they differ in the length of the swimming setae (longer in the Russian species). Bronstein's (1947) illustration of the hemipenis is also rather similar to the figures given by Külköylüoglu and Vinyard (2000). Smith et al. (2002) also noted that S. pseudobrowniana reported from Russia and Nevada are quite different from the European populations, and suggested that the respective material needs to be re-identified. We conclude that the

Russian and both North American records of S. pseudobrowniana represent new species and that the differences cannot be attributed to variability. This is supported by several reports of the absence of morphological variability among European populations of S. pseudobrowniana (see Kempf 1971, Danielopol and Vespremenau 1964, Petkovski 1966, Matzke-Karasz 1995, Meisch 2000).

## Key to genera and species of Scottiinae

1 First segment of the Mxl-palp with seven or eight setae, terminal segment of the Md-palp maximum 1.5 times longer than wide, "e" and " $f$ " setae on L6 long and equal 2

- $\quad$ First segment of the Mxl-palp with maximum five setae, terminal segment of the Md-palp at least two times longer than wide, "e" seta rarely long, and if so, " $f$ " seta equals half its length
2 Swimming setae on the A2 only slightly exceed distal end of the first endopodal segment
[non] Scot-
tia pseudobrowniana Kempf, 1971 in Külköylüoglu and Vinyard (2000)
- Swimming setae on the A2 longer. 3
3 Females in lateral view with dorsal margin evenly rounded towards both anterior and posterior ends, so that both ends equally wide and relatively narrow; males with inclined distal margin on the medial lobe of the lateral shield of the hemipenis towards dorsal side, and medial lobe of the lateral shield with a very short dorsal margin.... [non] Scottia pseudobrowniana Kempf, 1971 in Cole (1966)
- Females in lateral view with clearly wider posterior margin and the dorsal margin more broadly rounded towards posterior than towards anterior end; males with distal margin of the medial lobe rounded, but almost parallel to the rest of the hemipenis and this lobe with a longer dorsal margin 4
4 Medial lobe of the lateral shield on the hemipenis very narrow, almost finger like...... [non] Scottia pseudobrowniana Kempf, 1971 in Bronstein (1947)
- Medial lobe of the lateral shield rectangular- to square-shaped.................... 5

5 Dorsal lobe of the lateral shield on the hemipenis with a prominent projection.

Scottia birigida Smith et al., 2002

- No such projection present ........... Scottia pseudobrowniana Kempf, 1971

6 At least one of the UR posterior seta elongated and seta-like, reaching at least half of the posterior claw7

- This seta very short, thick and claw-like on both UR ..... 14
7 Penultimate segment on the L6 divided ..... 8
- Penultimate segment fused ..... 10
8 Seta "h3" on the L6 reaching or exceeding half the length of the terminalclaw9
- $\quad$ This seta at most reaching $1 / 3$ of the length of the terminal claw
Scottia insularis Chapman, 1963
- $\quad$ Posterior margin of the UR covered with small spine-like setae and carapace in lateral view more tumid
[non] Scottia
audax (Chapman, 1961) in De Deckker (1980) and Matzke-Karasz (1975) Four segments on the A1 fused, so that A1 is 5-segmented ........Austromesocypris berentsae Martens, De Deckker \& Rossetti, 2004 Only two segments on the A1 fused, so that A1 is 6-segmented. 11
11 One posterior seta on UR transformed into a thick claw, other more slender and seta-like.............. Austromesocypris tasmaniensis (De Deckker, 1983)
- Both posterior setae slender 12
12 Shell with straight dorsal margin, shape almost rectangular.
Austromesocypris bluffensis sp. n.
- Shell with gently rounded dorsal margin, shape more reniform ................ 13

13 Zenker organ with 10 rosettes of spines, hemipenis slender.... [non] Austromesocypris australiensis (De Deckker, 1983) in De Deckker (1983, Figure 5A-G)

- Zenker organ with more than 15 rosettes of spines, hemipenis robust.

Austromesocypris australiensis (De Deckker, 1983)
Seta "h3" on the L6 claw-like.
.Mesocypris pauliani Danielopol \& Betsch, 1980

- Seta "h3" on the L6 seta-like 15
15 Anterior seta on the UR short, not reaching $1 / 3$ the length of the anterior claw .....................................................Mesocypris pubescens Daday, 1910
- .................. This seta much longer, exceeding half the length of the anterior claw ........................................................................................................... 16
16 Seta " e " on the L6 long, almost reaching the distal end of the penultimate segment Mesocypris terrestris Harding, 1953
- Seta "e" on the L6 much shorter, not reaching middle of the penultimate segment .......... Mesocypris madagascariensis Danielopol \& Betsch, 1980


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## Appendix

List of ostracod species considered "terrestrial or semi-terrestrial" or often associated with those habitats

Superfamily Cypridoidea Baird, 1845
Family Cyprididae Baird, 1845
Subfamily Callistocypridinae Schornikov, 1980
Genus Callistocypris Schornikov, 1980
C. mckenzie Pinto, Rocha \& Martens, 2005: Saó Paolo State, Brazil (Pinto et al. 2005)
C. rossetti Pinto, Rocha \& Martens, 2005: Saó Paolo State, Brazil (Pinto et al. 2005)
C. zlotini Schornikov, 1980: Solomon Islands (Schornikov 1980)

Subfamily Cypridopsinae Kaufmann, 1900
Genus Bryocypris Røen, 1956
B. grandipes Røen, 1956: Cameroon (Martens 1989)

Subfamily Scottinae Bronstein, 1947
Tribe Mesocypridini Puri, 1974
Genus Austromesocypris Martens, De Deckker \& Rossetti, 2004
A. australiensis (De Deckker, 1983): Victoria, Australia (De Deckker 1983)
A. bluffensis sp. nov.: Tasmania (present paper)
A. berentsae Martens, De Deckker \& Rossetti, 2004: New South Wales, Australia (Martens et al. 2004)
A. tasmaniensis (De Deckker 1983): Tasmania, Australia (De Deckker 1983)

Genus Mesocypris Daday, 1910
M. madagascariensis Danielopol \& Betsch, 1980: Madagascar (Danielopol and Betsch 1980)
M. pauliani Danielopol \& Betsch, 1980: Madagascar (Danielopol and Betsch 1980)
M. pubescens Daday, 1910: Kilimanjaro (Daday 1910), Kenya (Klie 1939)
M. terrestris Harding, 1953: Malawi (Harding 1953)

Tribe Scottini Martens, De Deckker \& Rossetti, 2004
Genus Scottia Brady \& Norman, 1889
S. audax Chapman, 1961: New Zealand (Chapman 1961), Australia (De Deckker 1980, 1983)
S. birigida Smith, Matzke-Karasz, Kamiya \& Ikeda, 2002: Japan (Smith et al. 2002)
S. insularis Chapman, 1963: New Zealand (Chapman 1963)

## S. pseudobrowniana Kempf, 1971: Holarctic (Meisch 2000, Cole 1966, Külköylüo-

 glu and Vinyard 2000)Family Candonidae Kaufmann, 1900
Subfamily Candoninae Kaufmann, 1900
Tribe Terrestricypridini Schornikov 1980
Genus Caaporacandona Pinto, Rocha \& Martens, 2005
C. iguassuensis Pinto, Rocha \& Martens, 2005: Paraná State, Brazil (Pinto et al. 2005)
C. shornikovi Pinto, Martens \& Rossetti, 2005: Paraná State, Brazil (Pinto et al. 2005)

Genus Terrestricandona Danielopol \& Betsch, 1980
T. minuta Danielopol \& Betsch, 1980: Madagascar (Danielopol and Betsch 1980)

Genus Terrestricypris Schornikov, 1980
T. arborea Schornikov, 1980: Solomon Islands (Schornikov 1980)
T. wurdigae Pinto, Rocha \& Martens, 2005: Saō Paolo State, Brazil (Pinto et al. 2005)

Superfamily Cytheroidea Baird, 1850
Family Limnocytheridae Klie, 1938
Subfamily Timiriaseviinae Mandelstam, 1960
Genus Intrepidocythere Pinto, Rocha \& Martens, 2005
I. ibipora Pinto, Rocha \& Martens, 2005: Saó Paolo State, Brazil (Pinto et al. 2005)

Superfamily Terrestricytherioidea Schornikov, 1969
Family Terrestricytheridae Schornikov, 1969
Genus Terrestricythere Schornikov, 1969
T. elisabethae Horne, Smith, Whittaker \& Murray, 2004: England (Horne et al. 2004)
T. ivanovae Schornikov, 1969: Kuril Islands (Schornikov 1969)
T. pratensis Schornikov, 1980: Vladivostok, Russia (Schornikov 1980)
T. proboscidea Hiruta, Hiruta \& Mawatari, 2007 (Hiruta et al. 2007)

Superfamily Darwinuloidea Brady \& Norman, 1889
Family Darwinulidae Brady \& Norman, 1889
Genus Penthesilenula Rossetti \& Martens, 1998
P. aotearoa (Rossetti, Eagar \& Martens, 1998): Saó Paolo State, Brazil (Pinto et al. 2004, 2005)
P. brasiliensis (Pinto \& Kotzian, 1961): Saó Paolo State, Brazil (Pinto et al. 2004, 2005)
P. reidae Pinto, Rocha \& Martens, 2004: Saó Paolo State, Brazil (Pinto et al. 2004)

Genus Vestalenula Rossetti \& Martens, 1998
V. botocuda Pinto, Rocha \& Martens, 2003: Saó Paolo State, Brazil (Pinto et al. 2003)
V. irajai Pinto, Rocha \& Martens, 2003: Saó Paolo State, Brazil (Pinto et al. 2005)

# Two new species and new record of Batricavus Yin \& Li (Coleoptera, Staphylinidae, Pselaphinae) from China 

Zi-Wei Yin ${ }^{1, t,}$ Li-Zhen Li' ${ }^{1, \hbar}$<br>I Department of Biology, College of Life and Environmental Sciences, Shanghai Normal University, Shanghai, 200234, P. R. China<br>$\dagger$ urn:lsid:zoobank.org:author:C515106C-5782-4471-AE11-483B49606A67<br>$\ddagger$ urn:lsid:zoobank.org:author:BBACC7AE-9B70-4536-ABBE-54183D2ABD45<br>Corresponding author: Li-Zhen Li (lizhenli@shnu.edu.cn)

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urn:lsid:zoobank.org:pub:9B685A3C-6EB5-44F5-B463-ECA848BA5D51
Citation: Yin ZW, Li LZ (2012) Two new species and new record of Batricavus Yin \& Li (Coleoptera, Staphylinidae, Pselaphinae) from China. ZooKeys 215: 33-39. doi: 10.3897/zookeys.215.3570


#### Abstract

Two new species, Batricavus abdominalis Yin $\& \mathrm{Li}$, sp. n. and B. hainanensis Yin $\& \mathrm{Li}$, sp. n. are described from Hainan, South China, with male habitus and major diagnostic features illustrated. Batricavus tibialis Yin $\& \mathrm{Li}$ is newly recorded from Zhejiang, East China. Keys to both sexes of the genus are included.


## Keywords

Coleoptera, Staphylinidae, Pselaphinae, Batricavus, new species, new record, China, key

## Introduction

The monotypic genus Batricavus Yin $\& ~ L i ~ p r e s e n t l y ~ c o n t a i n s ~ o n l y ~ o n e ~ s p e c i e s, ~ B . ~ t i b i-~$ alis Yin \& Li from Guangdong, South China. The genus is separated from the allies primarily by the presence of a pair of anteropronotal foveiform pits in both sexes (for further discussion see Yin et al. 2011: 530). Recently, a series of Batricavus, representing two undescribed species, was collected during an expedition to Hainan Island. In additional, two male specimens representing a new record of B. tibialis, collected from South Zhejiang by Jiang-Qing Zhu, became available for study. This paper will
describe the new species, provide new distribution data for known species, and present identification keys to both males and females of the genus.

## Material and methods

Material treated in this study are housed in the Insect Collection of Shanghai Normal University, Shanghai, China (SNUC).

A slash $(/)$ is used to separate lines on the same label, a double slash $(/ /)$ is used to separate different labels. The terminology of the foveal system follows Chandler 2001, except for use 'ventrite' instead of 'sternite' when concerning the meso- and metathoracic structures.

The following acronyms are used in the text: BL - length of the body (= $\mathrm{HL}+\mathrm{PL}+\mathrm{EL}+\mathrm{AL}) ; \mathbf{H L}$ - length of the head from the anterior margin of the clypeus to the occipital constriction; HW - maximum width of the head across eyes; PL - length of the pronotum along the midline; PW - maximum with of the pronotum; EL length of the elytra along the suture; EW - maximum width of the elytra; AL - length of the abdomen along the midline; AW - maximum width of the abdomen.

## Taxonomy

## Key to males

1 Protibiae expanded laterally at apical 1/4 (Yin et al. 2011: 4, fig. 8) or near middle (Fig. 3A); metafemora and tergite IV simple 2

- Protibiae simple; metafemora broadened mesally at apical $1 / 4$ (Fig. 2B); tergite IV with large median cavity (Fig. 2A). (Hainan) ... B. abdominalis sp. $\mathbf{n}$.
2 Antennomeres VIII much larger than VII (Yin et al. 2011: 4, fig. 6); protibiae expanded laterally at apical $1 / 4$. (Guangdong, Zhejiang)
B. tibialis Yin \& Li
- Antennomeres VIII smaller than VII (Fig. 1B); protibiae expanded laterally near middle. (Hainan)
B. hainanensis sp. n.


## Key to females

1 Elytra and abdomen scattered with long setae except for normal pubescence..
B. abdominalis sp. n.

- Elytra and abdomen normally pubescent, lacking long setae ....................... 2

2 Head with basomedian carina extending anteriorly to level of posterior margin of vertexal foveae; tergite VIII with smooth posterior margin (Yin et al. 2011: 533, fig. 11)
B. tibialis Yin \& Li

- Head with basomedian carina extending anteriorly for very short distance, followed by short, thin sulcus reaching anteriorly and ending as small round pit at level of posterior margin of vertexal foveae; tergite VIII with serrate posterior margin (Figs 3D, E)
B. hainanensis sp. n.


## Batricavus abdominalis Yin \& Li, sp. n.

urn:lsid:zoobank.org:act:174178C3-F08F-4BD5-B9E9-EF2FAA2F120B
http://species-id.net/wiki/Batricavus_abdominalis
Figs 1A, 2

Type-locality. China, Hainan Province: Lingshui County, Diaoluoshan Natural Reserve, $18^{\circ} 43^{\prime} 36.93^{\prime \prime} \mathrm{N}, 109^{\circ} 52^{\prime} 55.21^{\prime \prime} \mathrm{E}, 950 \mathrm{~m}$ a.s.l.
 Diaoluoshan N. R. / road to reservoir / 25.iv.2012, $950 \mathrm{~m} /$ Pan et Li leg.'. Paratypes: $2 \widehat{\sigma}^{\top}, 8$ 아 , same label data as holotype; $1 \delta^{\lambda}, 1 q$, same label data except 'Zi-Wei Yin


Other material examined. $5 \widehat{\top} \widehat{o}^{\lambda}, 6 \uparrow \uparrow$, labeled 'China: Hainan Prov. / Ledong County / Jianfengling N. R. / 15.xi. 2005 / Jian-Hua Huang leg.'.

Diagnosis. Length 1.50-1.60; antennal club three-segmented, lacking obvious modification in male; male with simple protibiae and modified metafemora and tergite IV.

Description. Male. Length $1.50-1.59$; body (Fig. 1A) reddish brown, maxillary palpi and tarsi lighter. Head slightly wider than long, HL 0.34-0.35, HW 0.39-0.41. Eyes each composed of about 20 large facets. Antennal club three-segmented, segment VIII smallest, IX-XI enlarged. Pronotum rounded laterally, slightly wider than long, PL 0.38-0.40, PW 0.42-0.46. Elytra wider than long, EL $0.53-0.57$, EW 0.61-0.63, with scattered long setae. Protibiae simple; metafemora broadened mesally at apical 1/4 (Fig. 2B). Abdomen much narrower than elytra, AL 0.25-0.27, AW 0.53-0.55; tergite IV (Fig. 2A) with large median cavity comprising complicated structure. Aedeagus (Figs 2C, D) length 0.20, well-sclerotized, with elongate dorsal lobe.

Female. Measurements: BL 1.54-1.60, HL 0.33-0.34, HW 0.40-0.41, PL $0.37-$ 0.38 , PW 0.41-0.43, EL 0.50-0.53, EW 0.60-0.63, AL 0.34-0.35, AW 0.56-0.58.

Generally similar to male; eyes smaller, each composed of about 15 facets; tergite VIII (Fig. 2E) with smooth posterior margin; genital complex (Fig. 2F) transverse, width 0.24 , well sclerotized.

Distribution. South China: Hainan.
Etymology. The specific epithet refers to the male sexual character present on abdominal tergite IV.


Figure I. Male habitus of Batricavus A B. abdominalis B $B$. hainanensis. Scales: 0.5 mm .

## Batricavus hainanensis Yin \& Li, sp. n.

urn:lsid:zoobank.org:act:B16612EB-1867-40DA-9298-6A001C992718
http://species-id.net/wiki/Batricavus_hainanensis
Figs 1B, 3
Type-locality. China, Hainan Province: Lingshui County, Diaoluoshan Natural Reserve, $18^{\circ} 42^{\prime} 18.87^{\prime \prime} \mathrm{N}, 109^{\circ} 52^{\prime} 59.48^{\prime \prime} \mathrm{E}, 720 \mathrm{~m}$ a.s.l.
 aoluoshan N. R. / disc road / 26.iv.2012, 600-1,000 m / Dai et Peng leg.'. Paratypes: $1 \sigma^{\pi}, 2$ 아, same label data as holotype; $1 \circlearrowleft^{\top}$, same label data except 'road to reservoir / 25.iv.2012, 1,000 m'; 2 q $q$, same label data except 'Zi-Wei Yin leg'.

Other material examined. 1 , labeled 'China: Hainan Prov. / Jianfengling N. R. / Wufenqu area / 2.v.2012, alt. $900 \mathrm{~m} /$ Pan et Yin leg.'.

Diagnosis. Length 1.62-1.69; antennal club weakly indicated by apical three antennomeres, lacking obvious modification in male; male with modified protibiae and simple metafemora and tergite IV.


Figure 2. Diagnostic features of $B$. abdominalis $\mathbf{A}$ male tergite IV $\mathbf{B}$ male metafemur $\mathbf{C}$ aedeagus, in lateral view $\mathbf{D}$ same, in ventral view $\mathbf{E}$ female tergite VIII F female gential complex, in ventral view. Scales: $\mathrm{A}, \mathrm{B}=0.2 \mathrm{~mm}, \mathrm{C}-\mathrm{F}=0.1 \mathrm{~mm}$.


Figure 3. Diagnostic features of $B$. hainanensis $\mathbf{A}$ male protibia $\mathbf{B}$ aedeagus, in lateral view $\mathbf{C}$ same, in ventral view $\mathbf{D}$ female tergite VIII E same, posterior margin enlarged $\mathbf{F}$ female gential complex, in ventral view. Scales: $\mathrm{A}=0.2 \mathrm{~mm}, \mathrm{~B}-\mathrm{D}, \mathrm{F}=0.1 \mathrm{~mm}, \mathrm{E}=0.02 \mathrm{~mm}$.

Description. Male. Length 1.62-1.67; body (Fig. 1B) reddish brown, maxillary palpi and tarsi lighter. Head slightly wider than long, HL 0.32-0.33, HW 0.39-0.41. Eyes each composed of about 30 facets. Antennal club formed by apical three antennomeres, segment VIII smallest, IX-XI enlarged. Pronotum rounded laterally, about as long as wide, PL $0.39-0.40$, PW $0.40-0.41$. Elytra wider than long, EL $0.56-$ 0.58 , EW 0.63-0.64, covered by short pubescence. Protibiae (Fig. 3A) triangularly expanded laterally near middle; metafemora simple. Abdomen narrower than elytra, AL 0.35-0.36, AW 0.58-0.59; tergite IV simple in structure. Aedeagus (Figs 3B, C) length 0.18 , well-sclerotized, with elongate dorsal lobe.

Female. Measurements: BL 1.65-1.69, HL 0.34-0.35, HW 0.40-0.41, PL $0.38-$ 0.39 , PW 0.40-0.41, EL 0.53-0.54, EW 0.62-0.63, AL 0.40-0.41, AW 0.58-0.60.

Generally similar to male; eyes smaller, each composed of about 25 facets; tergite VIII (Fig. 3D, E) with serrate posterior margin; genital complex (Fig. 3F) transverse, width 0.27 , well sclerotized.

Distribution. South China: Hainan.
Etymology. The specific epithet refers to the type locality.

## Batricavus tibialis Yin \& Li, 2011

http://species-id.net/wiki/Batricavus_tibialis
Batricavus tibialis Yin \& Li, 2011: 532.
 Yun forest area / 7.v.2012, 1,200 m / Jian-Qing Zhu leg.'.

Distribution. South China: Guangdong; East China: Zhejiang (new provinc record).
Remarks. The two male specimens from Zhejiang are readily identified as B. tibialis because they have the male sexual characters (present on antennae and protibiae) and the aedeagal form perfectly agrees with those of the type series from Guangdong.

## Acknowledgment

We thank all the colleagues mentioned in the text for the collection and providing of the valuable specimens. The anonymous reviewer critically read the manuscript and provided helpful comments. The present study is supported by the National Science Foundation of China (No. 31172134) and Shanghai Normal University (Sk201242).

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Yin ZW, Li LZ, Zhao MJ (2011) Batricavus tibialis, a new genus and species of Batrisini from South China (Coleoptera: Staphylinidae: Pselaphinae). Acta Entomologica Musei Nationalis Pragae 51: 529-534.

# Two additional new species of the Stenus indubius group (Coleoptera, Staphylinidae) from China 

Liang Tang ${ }^{1, \dagger,}$ Li-Zhen $\mathrm{Li}^{1, \ddagger}$, Jun-Wang Wang ${ }^{2, \S}$<br>I Department of Biology, Shanghai Normal University, 100 Guilin Road, 1st Educational Building 323 Room, Shanghai, 200234 P. R. China 2 Qingliangfeng Nature Reserve administration, Changhua Town, Lin'an City, Zhejiang, 311321 P. R. China<br>$\dagger$ urn:lsid:zoobank.org:author:F45FE527-E59A-4702-A87E-B45BC33ED4C7<br>$\ddagger$ urn:lsid:zoobank.org:author:BBACC7AE-9B70-4536-ABBE-54183D2ABD45<br>§ urn:lsid:zoobank.org:author:A1E8FDBC-98D6-482F-99B3-DC49A79F9056<br>Corresponding author: Liang Tang (monkey_zzz1980@163.com)

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urn:lsid:zoobank.org:pub:5085EA0E-A234-4134-A39B-ED983D18D07C
Citation: Tang L, Li LZ, Wang JW (2012) Two additional new species of the Stenus indubius group (Coleoptera, Staphylinidae) from China. ZooKeys 215: 41-54. doi: 10.3897/zookeys.215.3702


#### Abstract

Two new species of the Stenus indubius group from China are described: S. huapingensis sp. n. from Guangxi Province and S. zhujianqingi sp. n. from Zhejiang Province. Habitus photos and illustrations of diagnostic characters of the new species and two described species, S. paradecens Tang \& Li, 2005 and $S$. guniujiangense Tang \& Li, 2005, are provided.


## Keywords

Coleoptera, Staphylinidae, Stenus indubius group, new species, China

## Introduction

After our recently published work (Tang and Li 2012) on Chinese species of the Stenus indubius group, we received new material containing two new species of the group collected from Guangxi and Zhejiang provinces, respectively. These new species are described in the present paper. One of them is closely related to $S$. paradecens Tang \&

[^0]Li, 2005 and S. guniujiangense Tang $\& \mathrm{Li}, 2005$. However, the figures in the original descriptions of the latter two species have minor flaws and are poorly printed. Therefore, new and improved illustrations are provided.

## Material and methods

The specimens examined in this paper were collected by sifting leaf litters in forests. For examination of the male genitalia, the last three abdominal segments were detached from the body after softening in hot water. The aedeagi, together with other dissected pieces, were mounted in Euparal (Chroma Gesellschaft Schmidt, Koengen, Germany) on plastic slides. Photos of sexual characters were taken with a Canon G7 camera attached to an Olympus SZX 16 stereoscope; habitus photos were taken with a Canon macro photo lens MP-E 65 mm attached to a Canon EOS60D camera.

The type specimens treated in this study are deposited in the following public and private collections:

SHNU Department of Biology, Shanghai Normal University, P. R. China cPut private collection V. Puthz, Schlitz, Germany

The measurements of proportions are abbreviated as follows:
BL body length, measured from the anterior margin of the clypeus to the posterior margin of abdominal tergite X
FL length of forebody, measured from the anterior margin of the clypeus to the apex of the elytra (apico-lateral angle)
HW width of head including eyes
PW width of pronotum
EW width of elytra
PL length of pronotum
EL length of elytra, measured from humeral angle apico-lateral angle
SL length of elytral suture

## Taxonomy

Stenus huapingensis Tang, Li \& Huang, sp. n.
urn:lsid:zoobank.org:act:FFC0FF4A-0EA5-4526-927F-1C3791815B75
http://species-id.net/wiki/Stenus_huapingensis
Figs 1, 5

Type material. Holotype $\delta^{\lambda}$ :"China: Guangxi Prov., Lingui County, Huaping N. R., Anjiangping, 1500m, 18.VII.2011, TANG Liang Leg." "Holotype / Stenus huapin-
gensis / Tang \& Li" [red handwritten label] (SHNU). Paratypes. 5 ふす, $12 q$ q, same locality but 1400-1700 m, 14-18 VII.2011, L. Tang, W.-J. He, Z. Peng, Y. Chen \& W.-L. Ma leg. (1 pair in cPut, remainder in SHNU).

Description. Brachypterous; body blackish, anterior margin of labrum brownish, antennae, maxillary palpi and legs yellowish brown, each elytron with an oval orange spot near lateral side, which is about $1 / 3$ as long as and $1 / 3$ as broad as the respective elytron.

BL: 4.1-4.9 mm; FL: 2.1-2.3 mm.
HW: $0.84-1.02 \mathrm{~mm}$, PL: $0.71-0.82 \mathrm{~mm}$, PW: $0.62-0.72 \mathrm{~mm}$, EL: $0.73-0.88$ mm , EW: $0.71-0.89 \mathrm{~mm}$, SL: $0.57-0.65 \mathrm{~mm}$.

Head 1.12-1.18 times as wide as elytra; interocular area with deep longitudinal furrows, median portion convex, extending to the level of inner eye margins; punctures round, well delimited in median portion and more or less confluent into diagonal stria in furrows, slightly larger and sparser in median area than near inner margins of eyes, diameter of large punctures about as wide as apical cross section of antennal segment II; interstices partially with faint reticulation, smaller than half the diameter of punctures except those in median portion and behind basi-antennal tubercles, which may be much larger. Antennae, when reflexed, extending a little beyond posterior margin of pronotum; relative length of antennal segments from base to apex as 11:8:19:11.5: 10: 9.5: 9.5: 6.5: 7: 8: 8.5. Paraglossa oval.

Pronotum 1.11-1.15 times as long as wide; disc with distinct median longitudinal furrow, two indistinct impressions in anterior half, indistinct transverse impression in the middle, and two indistinct impressions in posterior half; punctures moderately rugose and confluent, a little larger than those of head; interstices partially indistinctly reticulated, mostly smaller than half the diameter of punctures except those at the bottom of median longitudinal furrow, which may be distinctly larger.

Elytra 0.94-1.03 times as long as wide, lateral margins with slight concavity before the middle; disc slightly uneven with shallow longitudinal humeral impression, shallow postero-lateral impression, and distinct sutural impression; suture moderately convex; punctation and interstices similar to those of pronotum.

Hind tarsi 0.73 times as long as hind tibiae, tarsomeres IV distinctly bilobed.
Abdomen cylindrical; distinct paratergites absent, but rudimentary lateral border present; tergite VII with palisade fringe; punctures on abdominal tergites III-VIII round to elliptic, gradually becoming smaller posteriad; interstices smaller to little larger than half the diameter of punctures, with relatively faint reticulation on all $a b-$ dominal tergites.

Male. Sternite VII with posteromedian portion slightly flattened; sternite VIII (Fig. 5A) with shallow emargination at middle of posterior margin; sternite IX (Fig. 5B) with very long apico-lateral projections, posterior margin serrate; tergite X (Fig. 5C) with posterior margin slightly emarginated. Aedeagus (Figs 5D, 5E) with setae at sclerotized apex of median lobe; expulsion hooks (Fig. 5G) large; parameres extending beyond apex of median lobe, bisinuate, with about 13 setae on inner side (Fig. 5F).

Female. Abdomen broader than in male; sternite VIII (Fig. 5H) slightly prominent at the middle of posterior margin; tergite X (Fig. 5I) slightly emarginated at posterior margin; sclerotized spermatheca as in Figs 5J, 5K.

Distribution. China (Guangxi Province: Huaping Nature Reserve).
Biological notes. All the specimens were collected by shifting the mixture of bamboo leaves and broad tree leaves in dense forest.

Diagnoses. In general facies, the new species resembles S. zhaiyanbini Tang \& Li, 2012, but it may be distinguished by sparser and larger punctation of head, generally smaller elytral spots and the different sexual characters.

Etymology. The specific name is derived from "Huaping", the type locality of this species.

## Stenus zhujianqingi Tang, Li \& Huang, sp. n.

urn:lsid:zoobank.org:act:F6AE9DCA-B411-44C1-A181-E3B8DABC24E4
http://species-id.net/wiki/Stenus_zhujianqingi
Figs 2, 6

Type material. Holotype ${ }^{\text {J}}:$ "China: Zhejiang Prov., Lin'an City, Qingliangfeng N. R., 1750m, 9.VIII.2011, Zhu Jian-Qing leg." "Holotype / Stenus zhujianqingi / Tang \& Li" [red handwritten label] (SHNU). Paratypes. $13 \widehat{龴} \widehat{\jmath}, 2 q$, same data as for the holotype ( 1 pair in cPut, rest in SHNU); $4 \delta^{\lambda}, 1 q$, Qingliangfeng N. R., Longtangshan, $1100 \mathrm{~m}, 12$.V.2012, Chen, Ma \& Zhao leg. (SHNU).

Description. Brachypterous; body entirely black, anterior margin of labrum brownish, maxillary palpi yellowish brown, antennae and legs reddish brown.

BL: $4.0-4.3 \mathrm{~mm}$; FL: $2.0-2.2 \mathrm{~mm}$.
HW: $0.87-0.98 \mathrm{~mm}$, PL: $0.71-0.84 \mathrm{~mm}$, PW: $0.63-0.71 \mathrm{~mm}$, EL: $0.74-0.82$ mm , EW: $0.77-0.86 \mathrm{~mm}$, SL: $0.53-0.64 \mathrm{~mm}$.

Head 1.13-1.18 times as wide as elytra; interocular area with deep longitudinal furrows, median portion convex, not quite extending to level of inner eye margins; punctures round, mostly well delimited, slightly larger and sparser on median area than those near inner margins of eyes, diameter of large punctures about as wide as apical cross section of antennal segment II; interstices smooth, mostly much smaller than half the diameter of punctures. Antennae, when reflexed, not quite reaching posterior margin of pronotum; relative length of antennal segments from base to apex as 10.5: 7.5: 17.5: 10.5: 10: 7: 6.5: 5: 5: 5.5: 7.5. Paraglossa oval.

Pronotum 1.09-1.29 times as long as wide; disc with shallow short median longitudinal furrow, two indistinct impressions in anterior half, indistinct transverse impression in the middle, and two indistinct impressions in posterior half; punctures smaller than those of head, rugose and confluent; interstices smooth, slightly broader than diameter of punctures.

Elytra 0.95-1.00 times as long as wide, lateral margins with slight concavity before the middle; disc uneven with shallow longitudinal humeral impression, shallow sutural
impression and shallow postero-lateral impression; suture convex; punctation and interstices similar to those of pronotum, but more rugose and confluent.

Hind tarsi 0.72 times as long as hind tibiae, tarsomeres IV distinctly bilobed.
Abdomen cylindrical; segments III-VI with tergites and sternites completely fused, without paratergites or sutures; tergite VII with palisade fringe; punctures on abdominal tergites III-VIII round to elliptic, gradually becoming smaller posteriad, punctures of tergite III large, of similar size as those of head; interstices smooth, mostly smaller than half the diameter of punctures.

Male. Sternite VII with posteromedian portion slightly flattened; sternite VIII (Fig. 6A) with shallow emargination at middle of posterior margin; sternite IX (Fig. 6B) with very long apico-lateral projections, posterior margin serrate; tergite X (Fig. 6C) with posterior margin slightly emarginated. Aedeagus (Figs 6D, 6E) with minute setae at sclerotized apex of median lobe; expulsion hooks (Fig. 6G) very large; parameres extending distinctly beyond apex of median lobe, a little folded at apical third, with about 17 setae on inner side (Fig. 6F).

Female. Abdomen broader than in male; sternite VIII (Fig. 6H) inconspicuously prominent at middle of posterior margin; tergite X (Fig. 6I) broader than in male; sclerotized spermatheca as in Figs 6J, 6K.

Distribution. China (Zhejiang Province: Qingliangfeng N. R.).
Biological notes. All the specimens were collected by shifting leaves in coniferous and broad-leaved mixed forest.

Diagnoses. This species resembles S. paradecens Tang \& Li, 2005 and S. guniujiangensis Tang \& Li, 2005, but can be distinguished from both species by the rugose and confluent punctation of the pronotum and elytra. In addition, it also differs from $S$. paradecens by larger size and from S. guniujiangensis by longer elytra (see measurements in the modified key).

Etymology. This species is named in honor of Mr. Jian-Qing Zhu, who collected most of the specimens of the new species.

To accommodate the new species, the recently published key to the Chinese species of the Stenus indubius group (Tang and Li 2012) is modified at couplets 2 and 5 as follows:

2 Elytra shorter (EL/EW =0.86-0.93). Habitus: Fig. 4; sexual characters: Fig. 8. BL: 4.3-4.8 mm ............... S. guniujiangensis Tang \& Li. China: Anhui

- $\quad$ elytra longer (EL/EW $=0.95-1.01$ ) 2a
2a Body size larger (BL: 4.0-4.3 mm), punctation of pronotum and elytra rugose and confluent. Habitus: Fig. 2; sexual characters: Fig. 6 $\qquad$
S. zhujianqingi sp. n. China: Zhejiang
- Body size smaller (BL: 3.3-3.5 mm), punctation of pronotum and elytra well delimited. Habitus: Fig. 3; sexual characters: Fig. 7.
S. paradecens Tang \& Li. China: Anhui as the respective elytron. Habitus: Figs 7, 8 in Tang and Li (2012); sexual
characters: Figs 40-50 in Tang and Li (2012). BL: 4.3-4.7 mm $\qquad$
S. yinziweii Tang \& Li. China: Guizhou
- Elytral marks smaller, ranging from $1 / 3$ to $1 / 2$ as long and $1 / 3$ to $2 / 5$ as broad as the respective elytron...5a
5a Elytral marks on average smaller, about $1 / 3$ as long and $1 / 3$ as broad as the respective elytron; punctation of head sparser and coarser. Habitus: Fig. 1; sexual characters: Fig. 5. BL: 4.1-4.9 mm
S. huapingensis sp. n. China: Guangxi
- Elytral marks on average larger, ranging from $1 / 3$ to $1 / 2$ as long and $1 / 3$ to $2 / 5$ as broad as the respective elytron; punctation of head denser and finer. Habitus: Figs 9, 10 in Tang and $\operatorname{Li}$ (2012); sexual characters: Figs 51-61 in Tang and Li (2012). BL: $4.2-5.1 \mathrm{~mm}$
S. zhaiyanbini Tang \& Li. China: Guizhou

Figures


Figure I. Habitus of Stenus huapingensis in dorsal and ventral view. Scale $=1 \mathrm{~mm}$.


Figure 2. Habitus of Stenus zhujianqingi in dorsal and ventral view. Scale $=1 \mathrm{~mm}$.


Figure 3. Habitus of Stenus paradecens in dorsal and ventral view. Scale $=1 \mathrm{~mm}$.


Figure 4. Habitus of Stenus guniujiangense in dorsal and ventral view. Scale $=1 \mathrm{~mm}$.


Figures 5. Stenus huapingensis $\mathbf{A}$ male sternite VIII B male sternite IX C male tergites IX, X D, E aedeagus $\mathbf{F}$ apical portion of paramere $\mathbf{G}$ expulsion hooks $\mathbf{H}$ female sternite VIII I female tergites IX, X $\mathbf{J}$ valvifers and spermatheca $\mathbf{K}$ spermatheca. Scales $=0.25 \mathrm{~mm}$.


Figures 6. Stenus zhujianqingi $\mathbf{A}$ male sternite VIII B male sternite IX C male tergites IX, X D, E aedeagus $\mathbf{F}$ apical portion of paramere $\mathbf{G}$ expulsion hooks $\mathbf{H}$ female sternite VIII I female tergites IX, X $\mathbf{J}$ valvifers and spermatheca $\mathbf{K}$ spermatheca. Scales $=0.25 \mathrm{~mm}$.


Figures 7. Stenus paradecens $\mathbf{A}$ male sternite VIII B male sternite IX C male tergites IX, X D, E aedeagus $\mathbf{F}$ apical portion of paramere $\mathbf{G}$ expulsion hooks $\mathbf{H}$ female sternite VIII I female tergites IX, X J valvifers and spermatheca $\mathbf{K}$ spermatheca. Scales $=0.25 \mathrm{~mm}$.


Figures 8. Stenus guniujiangense $\mathbf{A}$ male sternite VIII B male sternite IX C male tergites IX, X D, E aedeagus $\mathbf{F}$ apical portion of paramere $\mathbf{G}$ expulsion hooks $\mathbf{H}$ female sternite VIII I female tergites IX, X $\mathbf{J}$ valvifers and spermatheca $\mathbf{K}$ spermatheca. Scales $=0.25 \mathrm{~mm}$.

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Tang L, Li L-Z (2012) Five new species of the Stenus indubius group (Coleoptera, Staphylinidae) from China. ZooKeys 165: 1-20. doi: 10.3897/zookeys.165.1773

# Aphanius arakensis, a new species of tooth-carp (Actinopterygii, Cyprinodontidae) from the endorheic Namak Lake basin in Iran 

Azad Teimori ${ }^{1,3, \dagger}$, Hamid Reza Esmaeili ${ }^{2, \ddagger}$, Zeinab Gholami ${ }^{1, \S}$, Neda Zarei ${ }^{2,1}$, Bettina Reichenbacher ${ }^{1, \pi}$<br>I Department of Earth- and Environmental Sciences, Palaeontology \& Geobiology \& GeoBio-CenterLMU, Ludwig-Maximilians-University, Richard-Wagner-Strasse 10, D-80333 Munich, Germany 2 Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran 3 Department of Biology, Faculty of Sciences, Shahid-Bahonar University of Kerman 22 Bahman Blvd. Kerman, 76169-14111 Iran<br>$\dagger$ urn:lsid:zoobank.org:author:8B247894-C118-465C-9054-92702C763BED<br>$\ddagger$ urn:lsid:zoobank.org:author:FF97C8FC-2D1B-4E53-A7E3-65098FFE98B2<br>§ urn:lsid:zoobank.org:author:8968FDD9-3CCF-4848-9E40-FFB41C1DC045<br>| urn:lsid:zoobank.org:author:666B6C38-2363-423F-8FA1-69D5E17B82E5<br>II urn:lsid:zoobank.org:author:224C51D4-0FE2-4253-BD9B-498FC3831F51<br>Corresponding author: Azad Teimori (a.teimori@lrz.uni-muenchen.de)

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#### Abstract

A new species of tooth-carp, Aphanius arakensis sp. n., is described from the Namak Lake basin in Iran. The new species is distinguished by the congeners distributed in Iran by the following combination of characters: 10-12 anal fin rays, 28-32 lateral line scales, 10-13 caudal peduncle scales, $8-10$ gill rakers, $12-19$, commonly $15-16$, clearly defined flank bars in males, a more prominent pigmentation along the flank added by relatively big blotches in the middle and posterior flank segments in females, a short but high antirostrum of the otolith that has a wide excisura, and a ventral rim with some small, drop-like processes, and 19 molecular apomorphies ( 17 transitions, two transversions) in the cytochrome $b$ gene. It was suggested based on the phylogenetic analysis that the new species is sister to $A$. sophiae from the Kor River and that $A$. farsicus from the Maharlu Lake basin is sister to $A$. arakensis plus $A$. sophiae. A noticeable feature of the Aphanius diversity in Iran is the conservatism of the external morphology as well as mor-


phometric and meristic characters, while distinctive differences are present in genetic characters, otolith morphology, and male color pattern. Transformation of the latter was probably driven by sexual selection.

## Keywords

Male color patterm, freshwater fish, tooth-carp, biodiversity, evolution, sexual selection

## Introduction

Aphanius is the only representative of the Cyprinodontidae (Teleostei, Cyprinodontiformes) in Eurasia. The genus occurs in coastal (brackish) and landlocked (freshwater to saline) water bodies in the Mediterranean and Persian Gulf basins from Iberian Peninsula as far eastwards as Iran and Pakistan (Wildekamp 1993). Aphanius species diversity is highest in the endorheic basins of the mountainous regions of central Anatolia and the Iranian plateau (Coad 2000; Hrbek and Meyer 2003, Hrbek et al. 2006, Esmaeili et al. 2012). Though central Anatolia is believed to represent the center of Aphanius speciation (Wildekamp et al. 1999), a high number of Aphanius species also occurs in Iran. Apart from the widely distributed $A$. dispar (Rüppell, 1829), seven endemic Aphanius species have been described from Iran to date, namely $A$. ginaonis (Holly, 1929) from the Genow hot spring near the Persian Gulf; A. isfahanensis Hrbek, Keivany \& Coad, 2006 from the endorheic Esfahan basin; A. farsicus Teimori, Esmaeili and Reichenbacher, 2011 from the endorheic Maharlu Lake basin [A. farsicus is a replacement name for the previous $A$. persicus (Jenkins, 1910) because this name has been recognized as a homonym of the fossil $A$. persicus (Priem, 1908) (Gaudant 2011, Teimori et al. 2011)]; A. sophiae (Heckel, 1849) from the endorheic Kor River Basin; A. vladykovi Coad, 1988 from the upper reaches of the Karoun basin; A. mesopotamicus Coad, 2009 from the Tigris-Euphrates drainage; and the recently re-established $A$. pluristriatus (Jenkins, 1910) from the Mond River drainage. In addition to the species listed above, Lebias punctatus and Lebias crystallodon were originally described from the Nemek Deria near Shiraz by Heckel (1846-1849). Berg (1949) and Coad (1996) considered L. punctatus to be a synonym of A. sophiae but at that time most of now valid species distributed in Iran were thought to be synonyms of the widely distributed $A$. sophiae. Coad (1996) strongly suggested that the type locality of $L$. punctatus is not the Lake Maharlu but some other lake nearby as a name Nemek Deria is a very common name in Farsi for a salt lake. However, later, the Kotschy's itinerary in southern Iran in 1841 and 1842 was studied in detail based on botanical labels and it was clearly shown that collections by Kotschy studied by Heckel indeed came from a lake now called Maharlu (Edmondson and Lack 2006). This aspect is not in the focus of this very paper; we tentatively consider L. punctatus to be a synonym of $A$. sophiae until a proper examination of the extant syntypes of Lebias punctatus is done.

A number of isolated Aphanius populations that might deserve species status have been reported from endorheic drainages in Iran, but have not yet been investigated in detail (Coad and Abdoli 2000; Hrbek et al. 2006; Esmaeili et al. 2010).


Figure I. Geographic distribution of the endemic Iranian inland Aphanius species.

They were commonly identified as A. sophiae (Heckel, 1849) (Coad and Abdoli 2000; Kamal et al. 2009); however, it was shown that the true A. sophiae is restricted to the endorheic Kor River basin near Shiraz (Fars Province) (Coad 2009; Esmaeili et al. 2012). This study describes a newly discovered Aphanius population from the Namak Lake basin in northern central Iran (Fig. 1). The specimens were collected in 2007 because they appeared to be different from other Iranian Aphanius species by a specific coloration. Here it is shown that the population from the Namak Lake basin in fact represents a new species, Aphanius arakensis. Our study is based on a total-evidence approach including morphometric and meristic characters, otolith morphology, and molecular data.

## Material and methods

Institutional acronyms: ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department; ZSM, Zoological State Collection, Munich.

## Material for morphological comparison

Aphanius sophiae: 35 males (19.3-33.3 mm SL) and 35 females (18.6-36.6 SL) from the Ghadamgah spring-stream system (close to type locality) in the Kor River Basin (Iran, Fars Province), $30^{\circ} 15^{\prime} \mathrm{N}, 52^{\circ} 25^{\prime}$ E. Males: ZM-CBSU, 8460, 8462, 8462, 8466, 8468, 8470, 8472-73, 8475, 8477, 8479, 8481, 8483, 8485, 8487, 8479, 8489, 8491-97, 8499, 8501, 8503-09, 8511-13; females: ZM-CBSU, 8461, 8463, 8465, 8467, 8469, 8471, 8474, 8476, 8478, 8480, 8482, 8484, 8486, 8488, 8490, 8498, 8500, 8502, 8510, 8514-29.
A. farsicus: 35 males (20.0-26.8 mm SL) and 35 females (20.4-35.2 mm SL) from the Barm-e-Shur spring in the Maharlu Lake Basin (type locality) (Iran, Fars Province), $29^{\circ} 27^{\prime} \mathrm{N}, 52^{\circ} 42^{\prime} \mathrm{E}$. Males: ZM-CBSU, 9413, 9415, 9417, 9421, 9441, 9443, 9447, 9449, 9459, 9467, 9481, 9483, 9485, 9487, 9489, 9493, 9497, 9499, 9503, 9511, 9513, 9515, 9517, 9519, 9527, 9529, 9531, 9533, 9537, 9539, 9541, 9555, 9557, 9559, 6375; females: ZM-CBSU, 9410, 9412, 9420, 9422, 9428, 9442, 9444, 9452, 9458, 9472, 9474, 9478, 9482, 9488, 9492, 9494, 9498, 9500, 9502, 9504, 9506, 9510, 9516, 9520, 9530, 9532, 9534, 9536, 9558, 9560, 9562, 9564, 6364, 6359, 6385.
A. isfahanensis: 18 males ( $17.6-23.8 \mathrm{~mm} \mathrm{SL}$ ) and 25 females (17.7-34.0 mm SL) from the Zayanderh River near Varzaneh, Esfahan Basin (type locality) (Iran, Esfahan Province), $32^{\circ} 25^{\prime} \mathrm{N}, 52^{\circ} 39^{\prime} \mathrm{E}$. Males: ZM-CBSU, 6472, 6474, 6476, 6478, 6480, 6482, 6484, 6486, 6488, 6490, 6492, 6494, 6496, 6498, 6500, 8602, 8604, 8613; females: ZM-CBSU, 6471, 6473, 6475, 6477, 6479, 6481, 6483, 6485, 6487, 6489, 6491, 6493, 6495, 6497, 6499 6501, 8603, 8605-8612.
A. vladykovi: 35 males (17.3-29.2 mm SL) and 35 females (16.1-41.4 mm SL) from the Chaghakhor wetland in the upper reaches of the Karoun Basin (Iran, Chahar Mahale Bakhtyari Province), $31^{\circ} 55^{\prime} \mathrm{N}, 50^{\circ} 56^{\prime} \mathrm{E}$. Males: ZM-CBSU, 640809, 6413-14, 6416, 6418, 6420-21, 6423, 6425-27, 6430, 6433-41, 6443-44, 6446, 6448-49, 6451-57: females: ZM-CBSU, 6401-03, 6405-07, 6410-12, $6415,6417,6419,6422,6424,6428-29,6431-32,6442,6445,6447,6450$, 6458-70.

## Material for molecular comparison

Aphanius sophiae ZM-CBSU, M46, M97, M98, M174-176 (Ghadamgah springstream system); A. farsicus ZM-CBSU, M47, M136, M177-178 (Barm-e-Shur spring); A. isfahanensis ZM-CBSU, M211, M213-214 (Zayanderh River near Varzaneh); A. arakensis sp. n. ZM-CBSU, M198-200 (Namak Lake Basin, 3400'N, 4950'E); A. vladykovi ZM-CBSU, M60, M139, M209 (Chaghakhor wetland in the upper reaches of the Karoun Basin).

Materials from GenBank. A. vladykovi: GenBank DQ367526; A. fasciatus: GenBank AF299273; A. iberus: GenBank AF299290. Poeciliopsis gracilis (GenBank AF 412155) was used as outgroup.

## Morphological analysis

Based on the morphometric schemes introduced in Holcik et al. (1989) and Doadrio et al. (2002), 18 morphometric parameters were measured using a Vernier calliper and recorded to the nearest 0.5 mm . The standard length was measured from the most anterior part of the snout to the base of the caudal fin rays. In total, 21 relative variables were calculated from the measurements (Table 1).

Scales removed from the left side of each fish, from the 3rd or 4th row below the dorsal fin, were mounted between microscope slides, and length and width of scales were measured to the nearest 0.1 mm by using a scale reader (Xerox 320). For each individual, scale length and scale width measurements were averaged to obtain a single length value and a single width value per individual and relative width and length of scales were calculated following Esmaeili (2001).

The meristic characters were counted under a stereomicroscope and consist of the numbers of (i) dorsal (ii) pectoral (iii) pelvic and (vi) anal fin rays, (v) lateral line series scales, (vi) caudal peduncle scales (the numbers of scales along the caudal peduncle, i.e. from the base of the last anal fin ray to the base of the caudal fin rays in a direct line), (vii) gill rakers and (viii) flank bars of males. Two posteriormost rays in dorsal and anal fins were calculated as one ray.

For examination of otolith morphology fish skulls were opened ventrally in order to remove the right and left otoliths. Otoliths were cleaned from tissue remains in $1 \%$ potassium hydroxide solution for $3-6 \mathrm{~h}$, washed several times and finally rinsed in distilled water for 12 h . Otolith morphology was analyzed under a stereo microscope. In addition, five or six otoliths from each population were examined by a scanning electron microscope (SEM) with a LEO 1430 VP at ZSM.

Univariate analysis of variance (ANOVA, with Duncan's post hoc test, $\mathrm{p}<0.05$ ) was used to test the significance of phenotypic differences among species and also between sexes. Canonical discriminant analysis (CDA) was used for multivariate analyses in order to document the classification success of the groups. The statistical analyses were carried out using PASW 19.00 (SPSS Inc 2011) and PAST (Hammer et al. 2001: PAlaeontological STatistics, version 1.81).

## Laboratory protocols and molecular analyses

Total genomic DNA was extracted according to phenol/chloroform procedures (Sambrook et al. 1989). A 900 base pairs (bp) fragment of the cytochrome b gene was successfully amplified via PCR using the primers (forward: Glu-F, 5' - AACCAC-
Table I. Morphometric characters of Aphanius arakensis sp. n. and other Iranian Aphanius species. Each cell contains mean $\pm$ standard deviation and range (mini-mum-maximum).

| Character | A. arakensis $\mathrm{n}=35$, male | A. arakensis $\mathrm{n}=35$, female | A. isfabanensis $\mathrm{n}=18$, male | A. isfahanensis $\mathrm{n}=25$, female | A. sophiae $\mathrm{n}=35$, male | $\begin{gathered} \text { A. sophiae } \\ \mathrm{n}=35, \\ \text { female } \end{gathered}$ | $\begin{gathered} \text { A. farsicus } \\ \text { n=35, } \\ \text { male } \end{gathered}$ | $\begin{gathered} \text { A. farsicus } \\ \text { n=35, } \\ \text { female } \end{gathered}$ | A. vladykovi $\mathrm{n}=35$, male | $\begin{gathered} \text { A. vladykovi } \\ \mathrm{n}=35, \\ \text { female } \\ \hline \end{gathered}$ | A. pluristriatus $\mathrm{n}=32$, male | A. pluristriatus $\mathrm{n}=38$, female |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| \% Standard length |  |  |  |  |  |  |  |  |  |  |  |  |
| Head length | $\begin{array}{r} 29.2 \pm 0.1 \\ (26.9-30.9) \\ \hline \end{array}$ | $\begin{gathered} 28.5 \pm 0.9 \\ (26.8-30.3) \end{gathered}$ | $\begin{gathered} 29.8 \pm 1.2 \\ (27.7-31.6) \\ \hline \end{gathered}$ | $\begin{gathered} 29.2 \pm 1.2 \\ (27.4-32.1) \\ \hline \end{gathered}$ | $\begin{gathered} 29.4 \pm 1.1 \\ (27.3-31.5) \\ \hline \end{gathered}$ | $\begin{gathered} 28.0 \pm 1.3 \\ (25.4-30.6) \end{gathered}$ | $\begin{gathered} 32.1 \pm 1.3 \\ (29.8-35.2) \end{gathered}$ | $\begin{array}{\|c\|} \hline 31.6 \pm 1.5 \\ (28.1-34.1) \\ \hline \end{array}$ | $\begin{gathered} 31.2 \pm 1.3 \\ (28.9-33.9) \\ \hline \end{gathered}$ | $\begin{gathered} 30.9 \pm 1.4 \\ (27.3-33.6) \\ \hline \end{gathered}$ | $\begin{gathered} 30.2 \pm 1.8 \\ (27.1-36.8) \\ \hline \end{gathered}$ | $\begin{gathered} 28.5 \pm 1.4 \\ (29.8-35.5) \\ \hline \end{gathered}$ |
| Head dep | $\begin{gathered} 22.1 \pm 0.8 \\ (20.0-23.9) \end{gathered}$ | $\begin{gathered} 21.5 \pm 0.8 \\ (20.0-23.5) \\ \hline \end{gathered}$ | $\begin{gathered} 20.9 \pm 1.0 \\ (18.9-22.3) \\ \hline \end{gathered}$ | $\begin{gathered} 20.2 \pm 1.0 \\ (17.8-22.3) \\ \hline \end{gathered}$ | $\begin{gathered} 21.9 \pm 0.8 \\ (19.3-23.9) \end{gathered}$ | $\begin{gathered} 21.1 \pm 1.12 \\ (18.8-23.9) \end{gathered}$ | $\begin{gathered} 23.9 \pm 1.4 \\ (20.8-26.1) \end{gathered}$ | $\begin{gathered} 22.9 \pm 1.3 \\ (20.0-25.0) \\ \hline \end{gathered}$ | $\begin{gathered} 23.7 \pm 0.9 \\ (21.9-26) \\ \hline \end{gathered}$ | $\begin{gathered} 23.2 \pm 1.2 \\ (20.5-25.7) \\ \hline \end{gathered}$ | $\begin{gathered} 21.6 \pm 1.1 \\ (19.7-24.5) \\ \hline \end{gathered}$ | $\begin{gathered} 19.8 \pm 0.9 \\ (23.3-21.2) \\ \hline \end{gathered}$ |
| Predorsal length | $\begin{gathered} 61.1 \pm 1.1 \\ (59.0-63.8) \end{gathered}$ | $\begin{gathered} 61.3 \pm 1.8 \\ (58.1-67.7) \end{gathered}$ | $\begin{gathered} 59.7 \pm 1.6 \\ (55.2-62.8) \end{gathered}$ | $\begin{gathered} 60.1 \pm 2.3 \\ (56.7-65.8) \end{gathered}$ | $\begin{gathered} 60.1 \pm 1.9 \\ (55.2-63.5) \end{gathered}$ | $\begin{gathered} 61.2 \pm 1.6 \\ (57.3-83.4) \end{gathered}$ | $\begin{gathered} 61.5 \pm 1.4 \\ (58.2-64.9) \end{gathered}$ | $\begin{gathered} 62.5 \pm 1.1 \\ (59.4-67.1) \end{gathered}$ | $\begin{gathered} 63.6 \pm 1.4 \\ (60.5-66.4) \end{gathered}$ | $\begin{gathered} 63.1 \pm 1.9 \\ (59.4-67.3) \end{gathered}$ | $\begin{gathered} 64.8 \pm 2.8 \\ (59.6-75.4) \end{gathered}$ | $\begin{gathered} 61.0 \pm 1.7 \\ (68.7-64.2) \end{gathered}$ |
| Length of pectoral fin | $\begin{gathered} 18.1 \pm 0.1 \\ (15.5-19.8) \end{gathered}$ | $\begin{gathered} 17.5 \pm 1.2 \\ (12.9-19.3) \end{gathered}$ | $\begin{gathered} \hline 16.4 \pm 1.3 \\ (12.9-19.0) \end{gathered}$ | $\begin{gathered} 16.2 \pm 1.2 \\ (13.9-18.2) \end{gathered}$ | $\begin{gathered} 18.3 \pm 1.1 \\ (15.8-20.1) \end{gathered}$ | $\begin{gathered} 17.0 \pm 1.3 \\ (14.9-19.6) \end{gathered}$ | $\begin{gathered} 19.4 \pm 1.2 \\ (17.1-21.8) \end{gathered}$ | $\begin{gathered} 17.2 \pm 1.1 \\ (15.0-19.4) \end{gathered}$ | $\begin{aligned} & 16.3 \pm 1.2 \\ & (14-20.3) \end{aligned}$ | $\begin{gathered} 15.4 \pm 1.1 \\ (13.5-17.6) \end{gathered}$ | $\begin{gathered} 18.6 \pm 1.8 \\ (14.6-22.1) \end{gathered}$ | $\begin{gathered} 17.8 \pm 1.3 \\ (14-20.4) \end{gathered}$ |
| Length of pelvic fin | $\begin{aligned} & 7.9 \pm 0.6 \\ & (6.6-9) \end{aligned}$ | $\begin{gathered} 7.9 \pm 0.5 \\ (7.1-9.3) \end{gathered}$ | $\begin{gathered} 6.7 \pm 0.7 \\ (5.7-8.4) \end{gathered}$ | $\begin{gathered} 6.6 \pm 0.8 \\ (5.1-7.1) \end{gathered}$ | $\begin{gathered} 10.1 \pm 0.8 \\ (8.7-11.7) \end{gathered}$ | $\begin{gathered} 8.7 \pm 0.8 \\ (6.9-10.3) \end{gathered}$ | $\begin{gathered} 8.6 \pm 0.8 \\ (6.7-10.2) \end{gathered}$ | $\begin{gathered} 7.6 \pm 0.9 \\ (5.7-9.2) \end{gathered}$ | $\begin{gathered} 6.7 \pm 0.7 \\ (5.4-8.1) \end{gathered}$ | $\begin{gathered} 6.5 \pm 0.8 \\ (4.5-8.0) \end{gathered}$ | $\begin{gathered} 7.4 \pm 1.3 \\ (4.7-11.0) \end{gathered}$ | $\begin{gathered} 6.7 \pm 0.6 \\ (5.0-8.0) \end{gathered}$ |
| Length of anal fin | $\begin{gathered} 12.5 \pm 1.7 \\ (9.8-14.3) \end{gathered}$ | $\begin{gathered} 11.7 \pm 1.2 \\ (10.4-13.4) \end{gathered}$ | $\begin{gathered} 11.4 \pm 2.4 \\ (9.6-12.7) \end{gathered}$ | $\begin{gathered} 10.7 \pm 2.8 \\ (8.7-13.3) \end{gathered}$ | $\begin{gathered} 13.4 \pm 1.7 \\ (11-16.1) \end{gathered}$ | $\begin{gathered} 11.8 \pm 1.9 \\ (14.1-11.8) \end{gathered}$ | $\begin{gathered} 13.4 \pm 2.6 \\ (10.7-17.2) \end{gathered}$ | $\begin{gathered} 11.8 \pm 2.1 \\ (9.7-13.3) \end{gathered}$ | $\begin{gathered} 12.8 \pm 1.0 \\ (10.6-15.2) \end{gathered}$ | $\begin{gathered} 11.7 \pm 2.0 \\ (9.3-16.8) \end{gathered}$ | $\begin{gathered} 13.7 \pm 1.0 \\ (11.5-15.3) \end{gathered}$ | $\begin{gathered} 12.5 \pm 1.1 \\ (8.9-14.5) \end{gathered}$ |
| Minimum body depth | $\begin{gathered} 16.5 \pm 0.6 \\ (15.1-17.7) \end{gathered}$ | $\begin{gathered} 15.2 \pm 0.7 \\ (13.8-16.5) \end{gathered}$ | $\begin{gathered} 14.8 \pm 0.6 \\ (14.0-15.8) \end{gathered}$ | $\begin{gathered} 14.4 \pm 0.8 \\ (12.4-15.6) \end{gathered}$ | $\begin{gathered} 16.6 \pm 1.1 \\ (14.3-18.9) \end{gathered}$ | $14.7 \pm 0.8$ $(13.3-16.8)$ | $\begin{gathered} 15.5 \pm 1.1 \\ (13.3-17.9) \end{gathered}$ | $\begin{gathered} 13.8 \pm 0.9 \\ (11.8-15.9) \end{gathered}$ | $\begin{gathered} 14.8 \pm 1.0 \\ (12.0-16.7) \end{gathered}$ | $\begin{gathered} 13.9 \pm 1.1 \\ (11.6-16.1) \end{gathered}$ | $\begin{gathered} 17.1 \pm 1.1 \\ (14.7-19.6) \end{gathered}$ | $\begin{gathered} 16.1 \pm 1.1 \\ (12.6-17.1) \end{gathered}$ |
| Pectoral anal fins distance | $\begin{gathered} 37.1 \pm 1.5 \\ (34.3-39.3) \end{gathered}$ | $\begin{gathered} 38.3 \pm 1.4 \\ (35.1-40.1) \end{gathered}$ | $\begin{gathered} 34.6 \pm 1.6 \\ (31.9-37.6) \end{gathered}$ | $\begin{gathered} 38.2 \pm 2.8 \\ (34.4-43.6) \end{gathered}$ | $\begin{gathered} 35.1 \pm 1.8 \\ (31.5-40.6) \end{gathered}$ | $\begin{gathered} 39.2 \pm 2.6 \\ (34.6-43.7) \end{gathered}$ | $\begin{gathered} 35.7 \pm 2.7 \\ (32.17-42) \end{gathered}$ | $\begin{gathered} 37.7 \pm 2.1 \\ (33.8-42.1) \end{gathered}$ | $\begin{gathered} 34.7 \pm 2.1 \\ (30-39.3) \end{gathered}$ | $\begin{gathered} 35.5 \pm 1.9 \\ (31.8-39.9) \end{gathered}$ | $\begin{gathered} 36.8 \pm 2.4 \\ (30.1-44.8) \end{gathered}$ | $\begin{gathered} 37.1 \pm 1.7 \\ (32.0-39.5) \end{gathered}$ |
| Pectoral pelvic fins distance | $\begin{gathered} 23.3 \pm 1.5 \\ (20.5-26.4) \end{gathered}$ | $\begin{gathered} 23.8 \pm 1.4 \\ (20.8-26.9) \end{gathered}$ | $\begin{gathered} 22.1 \pm 1.7 \\ (19.3-25.4) \end{gathered}$ | $\begin{gathered} 25.2 \pm 2.8 \\ (20.7-29.6) \end{gathered}$ | $21.1 \pm 1.1$ $(18.4-23.2)$ | $\begin{gathered} 23.5 \pm 2.5 \\ (18.6-28.0) \end{gathered}$ | $\begin{gathered} 22.8 \pm 2.6 \\ (18.1-30) \end{gathered}$ | $\begin{gathered} 24.1 \pm 2.1 \\ (19.6-28.6) \end{gathered}$ | $\begin{gathered} 22.2 \pm 2.1 \\ (17.8-25.9) \end{gathered}$ | $\begin{gathered} 22.7 \pm 1.6 \\ (19.5-25.9) \end{gathered}$ | $\begin{gathered} 24.1 \pm 2.2 \\ (20.1-31.5) \end{gathered}$ | $\begin{gathered} 23.6 \pm 1.7 \\ (18.4-26.7) \end{gathered}$ |
| Pelvic - anal fins distance | $\begin{gathered} 13.1 \pm 0.8 \\ (10.8-14.5) \end{gathered}$ | $\begin{gathered} 13.6 \pm 0.9 \\ (11.9-15.2) \end{gathered}$ | $\begin{gathered} 11.7 \pm 1.0 \\ (9.6-13.8) \end{gathered}$ | $\begin{gathered} 12.5 \pm 1.2 \\ (10.1-14.4) \end{gathered}$ | $\begin{gathered} 14.1 \pm 1.1 \\ (11.8-17.6) \end{gathered}$ | $\begin{gathered} 15.2 \pm 1.2 \\ (12.9-18.0) \end{gathered}$ | $\begin{gathered} 13.2 \pm 1.1 \\ (11.1-15.4) \end{gathered}$ | $\begin{gathered} 13.4 \pm 1.1 \\ (11.3-16.5) \end{gathered}$ | $\begin{gathered} 12.1 \pm 1.2 \\ (9.9-14.4) \end{gathered}$ | $\begin{gathered} 12.8 \pm 1.2 \\ (10.5-14.9) \end{gathered}$ | $\begin{gathered} 13.5 \pm 0.9 \\ (11.9-16.9) \end{gathered}$ | $\begin{gathered} 13 \pm 1.1 \\ (9.5-14.9) \end{gathered}$ |
| Length of caudal peduncle | $\begin{gathered} 22.1 \pm 0.8 \\ (19.9-23.9) \end{gathered}$ | $\begin{gathered} 21.9 \pm 0.7 \\ (20.0-23.2) \end{gathered}$ | $\begin{gathered} 23.7 \pm 1.3 \\ (21.6-27.2) \end{gathered}$ | $\begin{gathered} 22.8 \pm 1.1 \\ (20.0-24.4) \end{gathered}$ | $\begin{gathered} 22.8 \pm 1.4 \\ (19.7-25.7) \end{gathered}$ | $\begin{gathered} 23.4 \pm 1.7 \\ (19.6-26.9) \end{gathered}$ | $\begin{gathered} 21.7 \pm 1.3 \\ (18.9-23.9) \end{gathered}$ | $\begin{gathered} \hline 20.8 \pm 1.4 \\ (17.9-23.6) \end{gathered}$ | $\begin{gathered} 21.2 \pm 1.1 \\ (19.1-24.1) \end{gathered}$ | $\begin{gathered} 21.5 \pm 1.6 \\ (17.9-25.2) \end{gathered}$ | $\begin{gathered} \hline 22.8 \pm 1.3 \\ (19.6-25.7) \end{gathered}$ | $\begin{gathered} \hline 23.1 \pm 1.4 \\ (19.6-26.3) \end{gathered}$ |
| Length of caudal fin | $\begin{gathered} 20.3 \pm 1.5 \\ (14.9-23.1) \end{gathered}$ | $\begin{gathered} 19.5 \pm 0.1 \\ (16.8-21.6) \end{gathered}$ | $\begin{gathered} 20.2 \pm 1.9 \\ (17.4-24.3) \end{gathered}$ | $\begin{gathered} 19.2 \pm 1.4 \\ (16.4-21.3) \end{gathered}$ | $\begin{gathered} 20.1 \pm 1.3 \\ (17.7-23.3) \end{gathered}$ | $\begin{gathered} 18.1 \pm 1.6 \\ (14.9-22.6) \end{gathered}$ | $\begin{gathered} 21.7 \pm 1.0 \\ (17.8-24.6) \end{gathered}$ | $\begin{gathered} 19.1 \pm 1.3 \\ (16.7-21.6) \end{gathered}$ | $\begin{gathered} 20.3 \pm 1.25 \\ (17.5-22.5) \end{gathered}$ | $\begin{gathered} 19.2 \pm 1.4 \\ (16.2-22.3) \end{gathered}$ | $\begin{gathered} 20.4 \pm 2.3 \\ (15.8-26.4) \end{gathered}$ | $\begin{gathered} 19.7 \pm 1.2 \\ (13.3-22.7) \end{gathered}$ |
| Preanal distance | $\begin{gathered} \hline 68.4 \pm 1.3 \\ (66.3-71.2) \end{gathered}$ | $\begin{gathered} 69.2 \pm 0.1 \\ (66.7-71.7) \end{gathered}$ | $\begin{gathered} \hline 67.2 \pm 0.9 \\ (62.3-71.7) \end{gathered}$ | $\begin{gathered} 70.5 \pm 1.0 \\ (63.6-74.6) \end{gathered}$ | $\begin{gathered} 68.1 \pm 2.1 \\ (64.2-73.3) \end{gathered}$ | $\begin{gathered} 69.2 \pm 1.9 \\ (65.4-73.9) \end{gathered}$ | $\begin{gathered} 69.9 \pm 1.3 \\ (66.5-76.1) \end{gathered}$ | $\begin{gathered} 71.2 \pm 0.9 \\ (67.2-76.6) \end{gathered}$ | $\begin{gathered} 67.8 \pm 1.8 \\ (62.9-72.4) \end{gathered}$ | $\begin{gathered} 67.9 \pm 1.3 \\ (63.9-73.7) \end{gathered}$ | $\begin{gathered} 69.2 \pm 1.9 \\ (65.7-73.2) \end{gathered}$ | $\begin{gathered} 68.1 \pm 1.4 \\ (65.4-71.5) \end{gathered}$ |

Aphanius arakensis, a new species of tooth-carp (Cyprinodontidae)...

| Character | A. arakensis $\mathrm{n}=35$, male | A. arakensis $\mathrm{n}=35$, female | A. isfahanensis $\mathrm{n}=18$, male | A. isfahanensis $\mathrm{n}=25$, female | A. sophiae $\mathrm{n}=35$, male | A. sophiae $\mathrm{n}=35$, female | $\begin{gathered} \text { A. farsicus } \\ \text { n=35, } \\ \text { male } \end{gathered}$ | $\begin{array}{\|c\|} \hline \text { A. farsicus } \\ \text { n=35, } \\ \text { female } \\ \hline \end{array}$ | A. vadykovi $\mathrm{n}=35$, male | A. vladykovi $\mathrm{n}=35$, female | A. pluristriatus $\mathrm{n}=32$, male | $\underset{\substack{\text { A. pluristriatus } \\ \mathrm{n}=38, \text { female }}}{ }$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Scale length | $\begin{gathered} \hline 3.8 \pm 0.2 \\ (3.5-4.2) \\ \hline \end{gathered}$ | $\begin{aligned} & \hline 3.8 \pm 0.2 \\ & (3.3-4.4) \\ & \hline \end{aligned}$ | $\begin{gathered} \hline 3.1 \pm 0.2 \\ (2.7-3.5) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 3.2 \pm 0.3 \\ (2.6-4.0) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 3.1 \pm 0.3 \\ (2.7-3.7) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 3.1 \pm 0.3 \\ (2.6-3.8) \\ \hline \end{gathered}$ | $\begin{gathered} 3.5 \pm 0.4 \\ (2.7-4.5) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 3.4 \pm 0.4 \\ (2.5-4.6) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 2.6 \pm 0.3 \\ (1.9-3.3) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 2.4 \pm 0.3 \\ (1.8-3.2) \\ \hline \end{gathered}$ | $\begin{array}{r} 3.9 \pm 0.2 \\ (3.4-4.5) \\ \hline \end{array}$ | $\begin{aligned} & \hline 4.1 \pm 0.2 \\ & (3.8-4.5) \\ & \hline \end{aligned}$ |
| Scale width | $\begin{gathered} 3.9 \pm 0.2 \\ (3.5-3.4) \end{gathered}$ | $\begin{gathered} 3.8 \pm 0.2 \\ (3.3-4.6) \end{gathered}$ | $\begin{aligned} & 3.7 \pm 0.3 \\ & (3.3-4.3) \end{aligned}$ | $\begin{gathered} 3.8 \pm 0.4 \\ (3.1-4.9) \end{gathered}$ | $\begin{gathered} 3.4 \pm 0.4 \\ (2.8-4.18) \end{gathered}$ | $\begin{gathered} 3.3 \pm 0.4 \\ (2.6-4.1) \\ \hline \end{gathered}$ | $\begin{aligned} & 4.1 \pm 0.6 \\ & (3.2-5.4) \end{aligned}$ | $\begin{gathered} 3.9 \pm 0.5 \\ (3.1-4.9) \end{gathered}$ | $\begin{array}{r} 2.7 \pm 0.3 \\ (1.9-3.2) \\ \hline \end{array}$ | $\begin{gathered} 2.5 \pm 0.4 \\ (1.8-3.2) \end{gathered}$ | $\begin{gathered} 4.1 \pm 0.4 \\ (2.8-5.4) \end{gathered}$ | $\begin{gathered} 4.2 \pm 0.2 \\ (3.7-4.8) \end{gathered}$ |
| \% Preanal distance |  |  |  |  |  |  |  |  |  |  |  |  |
| Minimum body depth | $\begin{gathered} 24.1 \pm 0.1 \\ (21.7-25.9) \end{gathered}$ | $\begin{gathered} 22.0 \pm 0.8 \\ (20.2-23.4) \end{gathered}$ | $\begin{gathered} 22.0 \pm 1.3 \\ (19.7-24.5) \end{gathered}$ | $\begin{gathered} 20.1 \pm 1.3 \\ (18.3-23.1) \end{gathered}$ | $\begin{array}{\|c\|c\|} \hline 24.4 \pm 1.4 \\ (21.5-27.1) \end{array}$ | $\left.\begin{array}{\|c\|} \hline 21.3 \pm 1.1 \\ (19.3-23.3) \end{array} \right\rvert\,$ | $\begin{aligned} & 22.2 \pm 1.4 \\ & (19-25.1) \end{aligned}$ | $\begin{array}{c\|} \hline 19.4 \pm 1.4 \\ (16.8-22.8) \end{array}$ | $\begin{gathered} 21.9 \pm 1.6 \\ (17.6-24.8) \end{gathered}$ | $\begin{gathered} 20.5 \pm 1.7 \\ (16.9-23.5) \end{gathered}$ | $\begin{gathered} 24.5 \pm 1.3 \\ (21.2-27.1) \end{gathered}$ | $\begin{gathered} 23.7 \pm 1.6 \\ (18.8-26.5) \end{gathered}$ |
| Length of caudal peduncle | $\begin{gathered} 32.2+1.5 \\ (29.0-34.8) \end{gathered}$ | $\begin{gathered} 31.7 \pm 1.2 \\ (28.6-34.0) \end{gathered}$ | $\begin{gathered} 35.3 \pm 1.7 \\ (31.9-37.9) \end{gathered}$ | $\begin{gathered} 32.4 \pm 2.4 \\ (27.4-38.4) \end{gathered}$ | $\begin{gathered} \hline 33.6 \pm 2.4 \\ (27.9-38.7) \\ \hline \end{gathered}$ | $\begin{gathered} 33.9 \pm 3.1 \\ (27.2-40.3) \end{gathered}$ | $\begin{array}{c\|} \hline 31.1 \pm 2.5 \\ (25.1-35.7) \end{array}$ | $\begin{array}{\|c\|} \hline 29.3 \pm 2.5 \\ (23.9-33.8) \end{array}$ | $\begin{gathered} 31.3 \pm 1.8 \\ (28.0-35.5) \end{gathered}$ | $\begin{gathered} 31.6 \pm 2.7 \\ (26.1-38.1) \end{gathered}$ | $\begin{gathered} 33.1 \pm 2.2 \\ (27.4-37.8) \end{gathered}$ | $\begin{gathered} 33.8 \pm 2.3 \\ (28.2-38.1) \end{gathered}$ |
| Length of caudal fin | $\begin{gathered} 29.6 \pm 2.3 \\ (21.5-33.5) \end{gathered}$ | $\begin{gathered} 28.2 \pm 1.7 \\ (24.4-32.0) \end{gathered}$ | $\begin{gathered} 30.1 \pm 3.2 \\ (25.7-36.6) \end{gathered}$ | $\begin{gathered} 27.3 \pm 2.4 \\ (22.9-32.6) \end{gathered}$ | $\begin{gathered} 29.4 \pm 1.1 \\ (25.4-33.8) \end{gathered}$ | $\begin{array}{\|c\|} \hline 26.2 \pm 2.7 \\ (21.2-33.9) \end{array}$ | $\begin{gathered} 31.0 \pm 2.3 \\ (26.6-35.4) \end{gathered}$ | $\begin{gathered} 26.7 \pm 2.1 \\ (23.1-32.1) \end{gathered}$ | $\begin{gathered} 29.9 \pm 1.9 \\ (27.0-33.6) \end{gathered}$ | $\begin{gathered} 28.2 \pm 2.2 \\ (24.1-34.9) \end{gathered}$ | $\begin{gathered} 29.443 .0 \\ (23.2-36.6) \end{gathered}$ | $\begin{gathered} 28.1 \pm 2.9 \\ (19.5-33.3) \end{gathered}$ |
| Eye diameter | $\begin{gathered} 12.6 \pm 0.9 \\ (10.7-14.8) \end{gathered}$ | $\begin{gathered} 12.0 \pm 1.19 \\ (10.5-13.8) \end{gathered}$ | $\begin{gathered} 15.5 \pm 2.6 \\ (13.6-17.4) \end{gathered}$ | $\begin{gathered} 13.7 \pm 1.3 \\ (11.1-15.9) \end{gathered}$ | $\begin{gathered} \hline 13.7 \pm 0.9 \\ (11.5-14.9) \end{gathered}$ | $\begin{gathered} 12.7 \pm 1.1 \\ (10.0-15.3) \end{gathered}$ | $\begin{aligned} & 15.1 \pm 2.6 \\ & (13.4-17) \end{aligned}$ | $\left.\begin{array}{\|c\|} \hline 13.5 \pm 2.2 \\ (10.6-16.1) \end{array} \right\rvert\,$ | $\begin{array}{c\|} \hline 13.7 \pm 1.1 \\ (11.9-16.2) \end{array}$ | $\begin{gathered} 14.2 \pm 1.9 \\ (11.3-15.3) \end{gathered}$ | $\begin{gathered} 14.4 \pm 0.9 \\ (12.9-17.4) \end{gathered}$ | $\begin{gathered} 14.1 \pm 1.6 \\ (12.4-18.1) \end{gathered}$ |
| \% Head width |  |  |  |  |  |  |  |  |  |  |  |  |
| Interorbital distance | $\begin{aligned} & 1.1 \pm 0.06 \\ & (0.9-1.2) \end{aligned}$ | $\begin{gathered} 1.1 \pm 0.1 \\ (0.9-1.2) \end{gathered}$ | $\begin{gathered} 0.9 \pm 0.1 \\ (0.8-1) \end{gathered}$ | $\begin{gathered} 0.9 \pm 0.06 \\ (0.76-1.1) \end{gathered}$ | $\begin{aligned} & 0.9 \pm 0.06 \\ & (0.8-1.1) \end{aligned}$ | $\begin{gathered} 0.9 \pm 0.1 \\ (0.8-1.1) \end{gathered}$ | $\begin{gathered} 1.1 \pm 0.1 \\ (0.8-1.2) \end{gathered}$ | $\begin{gathered} 1.1 \pm 0.1 \\ (0.8-1.2) \end{gathered}$ | $\begin{aligned} & 0.8 \pm 0.05 \\ & (0.7-0.9) \end{aligned}$ | $\begin{aligned} & 0.8 \pm 0.06 \\ & (0.7-0.9) \end{aligned}$ | $\begin{aligned} & 0.9 \pm 0.05 \\ & (0.8-1.1) \end{aligned}$ | $\begin{aligned} & 1.0 \pm 0.06 \\ & (0.8-1.1) \end{aligned}$ |
| \% Head length |  |  |  |  |  |  |  |  |  |  |  |  |
| Eye diameter | $\begin{aligned} & 0.3 \pm 0.01 \\ & (0.2-0.3) \end{aligned}$ | $\begin{gathered} 0.3 \pm 0.2 \\ (0.2-0.3) \end{gathered}$ | $\begin{aligned} & 0.3 \pm 0.01 \\ & (0.3-0.4) \end{aligned}$ | $\begin{aligned} & 0.3 \pm 0.02 \\ & (0.3-0.4) \end{aligned}$ | $\begin{gathered} 0.3 \pm 0.02 \\ (0.3-0.35) \end{gathered}$ | $\begin{gathered} 0.3 \pm 0.02 \\ (0.2-0.35) \\ \hline \end{gathered}$ | $\begin{aligned} & 0.3 \pm 0.02 \\ & (0.3-0.4) \end{aligned}$ | $\begin{aligned} & 0.3 \pm 0.03 \\ & (0.2-0.3) \end{aligned}$ | $\begin{aligned} & 0.3 \pm 0.02 \\ & (0.2-0.3) \end{aligned}$ | $\begin{gathered} 0.3 \pm 0.1 \\ (0.2-1.2) \end{gathered}$ | $\begin{aligned} & 0.3 \pm 0.02 \\ & (0.3-0.4) \end{aligned}$ | $\begin{aligned} & 0.3 \pm 0.02 \\ & (0.3-0.4) \end{aligned}$ |
| \% Eye diameter |  |  |  |  |  |  |  |  |  |  |  |  |
| Preorbital distance | $\begin{gathered} 0.8 \pm 0.1 \\ (0.7-0.9) \end{gathered}$ | $\begin{gathered} 0.8 \pm 0.1 \\ (0.7-1.0) \end{gathered}$ | $\begin{aligned} & 0.7 \pm 0.05 \\ & (0.6-0.8) \end{aligned}$ | $\begin{gathered} 0.7 \pm 0.1 \\ (0.6-1.0) \end{gathered}$ | $\begin{gathered} 0.8 \pm 0.1 \\ (0.7-1.1) \end{gathered}$ | $\begin{gathered} 0.8 \pm 0.1 \\ (0.6-1.0) \end{gathered}$ | $\begin{gathered} 0.8 \pm 0.1 \\ (0.6-1.1) \end{gathered}$ | $\begin{aligned} & 0.8 \pm 0.08 \\ & (0.7-1.1) \end{aligned}$ | $\begin{gathered} 0.9 \pm 0.1 \\ (0.8-1.1) \end{gathered}$ | $\begin{gathered} 0.9 \pm 0.1 \\ (0.2-1.1) \end{gathered}$ | $\begin{aligned} & 0.8 \pm 0.05 \\ & (0.6-0.9) \end{aligned}$ | $\begin{gathered} 0.8 \pm 0.1 \\ (0.6-0.9) \end{gathered}$ |
| \% Minimum body depth |  |  |  |  |  |  |  |  |  |  |  |  |
| Length of caudal peduncle | $\begin{gathered} 1.3 \pm 0.1 \\ (1.2-1.5) \end{gathered}$ | $\begin{aligned} & 1.4 \pm 0.1 \\ & (1.3-1.6) \end{aligned}$ | $\begin{gathered} 1.6 \pm 0.1 \\ (1.5-1.9) \end{gathered}$ | $\begin{gathered} 1.6 \pm 0.1 \\ (1.3-1.8) \end{gathered}$ | $\begin{aligned} & 1.4 \pm 0.15 \\ & (1.1-1.7) \end{aligned}$ | $\begin{aligned} & 1.6 \pm 0.15 \\ & (1.3-2.0) \end{aligned}$ | $\begin{gathered} 1.4 \pm 1.5 \\ (1.1-1.8) \end{gathered}$ | $\begin{gathered} 1.5 \pm 0.1 \\ (1.2-1.9) \end{gathered}$ | $\begin{gathered} 1.4 \pm 0.1 \\ (1.2-1.7) \end{gathered}$ | $\begin{aligned} & 1.5 \pm 0.2 \\ & (1.1-1.9) \end{aligned}$ | $\begin{gathered} 1.3 \pm 0.1 \\ (1.2-1.6) \end{gathered}$ | $\begin{aligned} & 1.4 \pm 0.2 \\ & (1.2-1.6) \end{aligned}$ |

CGTTGTATTCAACTACAA-3'; reverse: ThrR, 5’-CCTCCGATCTTCGGATTA-CAAGACCG-3' (Machordom and Doadrio 2001). Amplification was performed in a thermal cycler programmed as follows: initial $94^{\circ} \mathrm{C}$ for $3 \mathrm{~min}, 35$ cycles at $94^{\circ} \mathrm{C}$ for $50 \mathrm{~s}, 56^{\circ} \mathrm{C}$ for $45 \mathrm{~s}, 72^{\circ} \mathrm{C}$ for 1 min , followed by a final extension at $72^{\circ} \mathrm{C}$ for 5 min. Sequencing was performed by Macrogen company, South Korea. Cytochrome $b$ nucleotide sequences were edited with BioEdit and aligned through Geneious pro v5.4 (Drummond et al. 2011). Additional Aphanius sequences were obtained from the NCBI GenBank (http://www.ncbi.nlm.nih.gov) and included in the analyses (see above). The achieved cytb sequences for the here studied Aphanius populations were deposited in GenBank under numbers JX154880-JX154898.

Maximum likelihood-based phylogenetic relationships were estimated by using the program SeaView version 4 (Gouy et al. 2010). The best-fit model of nucleotide substitution was obtained using the program JmodelTest 0.1.1 (Posada 2008). Accordingly, the GTR + I + G model (= General Time Reversible model + proportion of Invariable sites + Gamma-shaped distribution of rates across sites) was chosen.

Maximum parsimony based phylogenetic relationships were estimated using the program SeaView version 4 (Gouy et al. 2010) with 100 heuristic searches using random additions of sequences and implementing the Close-Neighbor-Interchange (CNI) on random tree algorithm. To test this phylogeny, bootstrap method using 2000 replication was used. To document the degree of homoplasy and degree to which potential synapomorphy is exhibited on the tree, the Consistency Index (CI) and the Retention Index (RI) were calculated by using the parsimony model within the Mesquite system for phylogenetic computing (Maddison and Maddison 2011).

The Neighbor Joining (NJ) distance-based phylogenetic relationships were estimated by using the computer program Geneious pro v5.4 (Drummond et al. 2011). The HKY85 model (Hasegawa et al. 1985) of molecular evolution was used with gamma distributed among site rate variation. There were a total of 771 positions in the final dataset.

## Results

## Aphanius arakensis sp. n.

urn:lsid:zoobank.org:act:D9995F4C-AF0A-4791-9D80-D759EFEDA569
http://species-id.net/wiki/Aphanius_arakensis
Figure 2A, B

Holotype. Male, 38.5 mm TL, 31.5 mm SL, Iran, Arak, Namak Lake Basin, $34^{\circ} 00^{\prime} \mathrm{N}$; $49^{\circ} 50^{\prime}$ E, Altitude 1786 m, 26 September 2007, A. Teimori, M. Ebrahimi, A. Gholamifard and A. Gholmhosseini (ZM-CBSU 10999).

Paratypes. 35 males ( $22.6-32.7 \mathrm{~mm} \mathrm{SL}$ ), 35 females ( $22.5-34.1 \mathrm{~mm} \mathrm{SL}$ ), same locality as holotype (ZM-CBSU 11000, 11051-11118).

Diagnosis. The new species is distinguished by the congeners distributed in Iran by the following combination of characters: 10-12 anal fin rays, 28-32 lateral line


Figure 2. A Aphanius arakensis, holotype, male, 31.5 mm SL (ZM-CBSU 10999) B paratype, female, 31.5 mm SL (ZM-CBSU 11054).
scales, 10-13 caudal peduncle scales, $8-10$ gill rakers, 12-19, commonly 11-13, clearly defined flank bars in males, a more prominent pigmentation along the flank added by relatively big blotches in the middle and posterior flank segments in females, a short but high antirostrum of the otolith that has a wide excisura, and a ventral rim with some small, drop-like processes and 19 molecular apomorphies ( 17 transitions, two transversions) in the cytochrome $b$ gene.

Description of the holotype. The males of the new species reach approximately 32 mm SL and have 12-19 flank bars, the females are usually larger than the males and reach approximately 34 mm SL.

The morphometric characters are summarized in Table 1. Compared to the other examined Aphanius species, A. arakensis sp. n. shows higher mean values of the minimum body depth, width and length of scales, distances between the pectoral and pelvic fins and the interorbital distance, but significantly lower mean values for the eye diameter and the caudal peduncle length (differences are statistically significant, $\mathrm{p}<0.05$ ).

The meristic characters are summarized in Table 2. The dorsal fin is characterized by a somewhat curved superior border, and has 11-14 rays; the anal fin shows a round superior border and includes $10-12$ rays; the pectoral fin is rounded and consists of $14-18$ rays; the pelvic fin is relatively short, positioned just anteriorly to the anal fin and comprises 6-8 rays. The caudal fin is rounded; the caudal peduncle possesses $10-13$ scales. The number of lateral line series scales is $27-32$. However, the ANOVA analysis reveals that only the numbers of lateral line series scales and caudal
Table 2. Meristic characters (mean $\pm$ standard deviation and range) of Iranian Aphanius species.

| Character | A. arakensis $\mathrm{n}=35$, male | A. arakensis $\mathrm{n}=35$, female | A. isfahanensis $\mathrm{n}=18$, male | A. isfabanensis $\mathbf{n}=25$, female | A. sophiae $\mathrm{n}=35$, male | $\begin{gathered} \text { A. sophiae } \\ \mathrm{n}=35, \\ \text { female } \end{gathered}$ | $\begin{gathered} \text { A. farsicus } \\ \mathrm{n}=35, \\ \text { male } \end{gathered}$ | $\begin{gathered} \text { A. farsicus } \\ \mathrm{n}=35, \\ \text { female } \end{gathered}$ | A. vladykovi $\mathrm{n}=35$, male | A. vladykovi $\mathrm{n}=35$, female | A. pluristriatus $\mathrm{n}=32$, male | A. pluristriatus $\mathrm{n}=38$, female |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Dorsal fin rays | $\begin{aligned} & \hline 12.2 \pm 0.8 \\ & (11-14) \end{aligned}$ | $\begin{aligned} & 12.3 \pm 0.7 \\ & (11-14) \end{aligned}$ | $\begin{aligned} & \hline 11.7 \pm 0.6 \\ & (11-13) \end{aligned}$ | $\begin{gathered} \hline 11.6 \pm 0.7 \\ (10-13) \end{gathered}$ | $\begin{aligned} & \hline 13.7 \pm 0.6 \\ & (13-15) \end{aligned}$ | $\begin{gathered} 13.8 \pm 0.8 \\ (13-15) \end{gathered}$ | $\begin{aligned} & \hline 12.1 \pm 0.8 \\ & (11-14) \end{aligned}$ | $\begin{aligned} & 12 \pm 0.8 \\ & (10-13) \end{aligned}$ | $\begin{gathered} 13.2 \pm 0.8 \\ (12-15) \end{gathered}$ | $\begin{aligned} & 13.5 \pm 0.7 \\ & (12-15) \end{aligned}$ | $\begin{aligned} & 13.8 \pm 0.7 \\ & (12-15) \end{aligned}$ | $\begin{aligned} & 13.6 \pm 0.7 \\ & (12-15) \end{aligned}$ |
| Pectoral fin rays | $\begin{aligned} & 16.7 \pm 0.9 \\ & (14-18) \end{aligned}$ | $\begin{aligned} & 16.8 \pm 0.6 \\ & (16-18) \end{aligned}$ | $\begin{aligned} & 15.9 \pm 0.8 \\ & (15-17) \end{aligned}$ | $\begin{gathered} 16.3 \pm 0.5 \\ (15-17) \end{gathered}$ | $\begin{aligned} & 18 \pm 0.8 \\ & (16.20) \end{aligned}$ | $\begin{gathered} 17.9 \pm 0.7 \\ (17-19) \end{gathered}$ | $\begin{gathered} 15.4 \pm 0.7 \\ (14-17) \end{gathered}$ | $\begin{aligned} & 15.4 \pm 0.6 \\ & (14-17) \end{aligned}$ | $\begin{aligned} & 16.5 \pm 0.8 \\ & (14-18) \end{aligned}$ | $\begin{aligned} & 16.6 \pm 0.7 \\ & (15-18) \end{aligned}$ | $\begin{gathered} 17.1 \pm 0.7 \\ (16-19) \end{gathered}$ | $\begin{aligned} & 17 \pm 0.6 \\ & (16-18) \end{aligned}$ |
| Pelvic fin rays | $\begin{gathered} 7.3 \pm 0.5 \\ (6-8) \end{gathered}$ | $\begin{gathered} 7.2 \pm 0.4 \\ (7-8) \end{gathered}$ | $\begin{aligned} & 7 \pm 0.6 \\ & (6-8) \end{aligned}$ | $\begin{gathered} 6.9 \pm 0.5 \\ (6-8) \end{gathered}$ | $\begin{gathered} 7.3 \pm 0.5 \\ (6-8) \end{gathered}$ | $\begin{gathered} 7.3 \pm 0.6 \\ (6-8) \end{gathered}$ | $\begin{gathered} 6.7 \pm 0.4 \\ (6-7) \end{gathered}$ | $\begin{gathered} 6.6 \pm 0.5 \\ (5-7) \end{gathered}$ | $\begin{aligned} & 7 \pm 0.6 \\ & (6-9) \end{aligned}$ | $\begin{gathered} 6.9 \pm 0.4 \\ (6-8) \end{gathered}$ | $\begin{gathered} 7.2 \pm 0.5 \\ (6-8) \end{gathered}$ | $\begin{gathered} \hline 7.1 \pm 0.4 \\ (8-7) \end{gathered}$ |
| Anal fin rays | $\begin{aligned} & 11.4 \pm 0.5 \\ & (10-12) \end{aligned}$ | $\begin{aligned} & 11.5 \pm 0.5 \\ & (11-12) \end{aligned}$ | $\begin{aligned} & 10.9 \pm 0.3 \\ & (10-11) \end{aligned}$ | $\begin{aligned} & 11.1 \pm 0.5 \\ & (10-12) \end{aligned}$ | $\begin{aligned} & 12.3 \pm 0.6 \\ & (11-14) \end{aligned}$ | $\begin{gathered} 12.7 \pm 1.1 \\ (12-17) \end{gathered}$ | $\begin{aligned} & 11.1 \pm 0.6 \\ & (10-12) \end{aligned}$ | $\begin{aligned} & 11.1 \pm 0.7 \\ & (10-12) \end{aligned}$ | $\begin{gathered} 13.2 \pm 0.7 \\ (12-15) \end{gathered}$ | $\begin{aligned} & 13.2 \pm 0.8 \\ & (12-15) \end{aligned}$ | $\begin{aligned} & 13 \pm 0.7 \\ & (12-14) \end{aligned}$ | $\begin{gathered} 12.5 \pm 0.6 \\ 11-14 \end{gathered}$ |
| Lateral line series scales | $\begin{gathered} 30.1 \pm 1.0 \\ (29-32) \end{gathered}$ | $\begin{gathered} 29.6 \pm 1.1 \\ (28-32) \end{gathered}$ | $\begin{gathered} 24.9 \pm 1.5 \\ (23-27) \end{gathered}$ | $\begin{aligned} & \hline 26 \pm 1.4 \\ & (23-27) \end{aligned}$ | $\begin{gathered} 27.9 \pm 0.9 \\ (26-29) \end{gathered}$ | $\begin{gathered} 27.1 \pm 1.3 \\ (25-29) \end{gathered}$ | $\begin{gathered} 25.6 \pm 1.6 \\ (22-28) \end{gathered}$ | $\begin{aligned} & 25.4 \pm 1.5 \\ & (23-29) \end{aligned}$ | $\begin{gathered} 36.3 \pm 2.8 \\ (33-43) \end{gathered}$ | $\begin{gathered} 37.1 \pm 2.7 \\ (33-43) \end{gathered}$ | $\begin{aligned} & 27.1 \pm 1.1 \\ & (24-29) \end{aligned}$ | $\begin{aligned} & 27 \pm 1.2 \\ & (24-29) \end{aligned}$ |
| Caudal peduncle scales | $\begin{aligned} & 11.6 \pm 0.6 \\ & (10-13) \end{aligned}$ | $\begin{aligned} & 11.6 \pm 0.7 \\ & (10-13) \end{aligned}$ | $\begin{aligned} & 10 \pm 0.5 \\ & (9-11) \end{aligned}$ | $\begin{gathered} 10.3 \pm 0.8 \\ (9-12) \end{gathered}$ | $\begin{gathered} 9.9 \pm 0.8 \\ (8-11) \end{gathered}$ | $\begin{gathered} 9.7 \pm 0.6 \\ (9-11) \end{gathered}$ | $\begin{gathered} 9.26 \pm 0.6 \\ (8-11) \end{gathered}$ | $\begin{aligned} & 9.4 \pm 0.6 \\ & (8-10) \end{aligned}$ | $\begin{gathered} 12.6 \pm 1.1 \\ (10-14) \end{gathered}$ | $\begin{gathered} 12.5 \pm 1.4 \\ (9-15) \end{gathered}$ | $\begin{gathered} 9.2 \pm 0.7 \\ (8-11) \end{gathered}$ | $\begin{gathered} 9.3 \pm 0.8 \\ (8-11) \end{gathered}$ |
| Gill raker | $\begin{aligned} & 9.2 \pm 0.5 \\ & (8-10) \\ & \hline \end{aligned}$ | $\begin{aligned} & 9.3 \pm 0.5 \\ & (8-10) \\ & \hline \end{aligned}$ | $\begin{aligned} & 10.8 \pm 0.5 \\ & (10-12) \\ & \hline \end{aligned}$ | $\begin{gathered} 11.1 \pm 0.7 \\ (10-13) \\ \hline \end{gathered}$ | $\begin{gathered} 10.7 \pm 0.7 \\ (9-12) \\ \hline \end{gathered}$ | $\begin{gathered} 10.7 \pm 0.8 \\ (9-12) \\ \hline \end{gathered}$ | $\begin{gathered} 10.9 \pm 0.7 \\ (9-13) \\ \hline \end{gathered}$ | $\begin{gathered} 10.7 \pm 0.8 \\ (9-12) \\ \hline \end{gathered}$ | $\begin{aligned} & 9.7 \pm 0.1 \\ & (8-12) \\ & \hline \end{aligned}$ | $\begin{aligned} & 9.7 \pm 0.7 \\ & (8-11) \end{aligned}$ | $\begin{gathered} 9.8 \pm 0.6 \\ (8-11) \\ \hline \end{gathered}$ | $\begin{gathered} 9.6 \pm 0.7 \\ (8-11) \\ \hline \end{gathered}$ |
| Flank bars | $\begin{aligned} & 15.9 \pm 1.4 \\ & (12-19) \end{aligned}$ | - | $\begin{gathered} 10.7 \pm 0.9 \\ (9-13) \end{gathered}$ | - | $\begin{gathered} 11.9 \pm 1.5 \\ (8-15) \end{gathered}$ | - | $\begin{gathered} 12.4 \pm 1.2 \\ (10-16) \end{gathered}$ | - | $\begin{aligned} & 11 \pm 1.2 \\ & (8-13) \end{aligned}$ | - | $\begin{gathered} 13.8 \pm 1.7 \\ (11-17) \end{gathered}$ | - |

peduncle scales (in males and females), as well as the numbers of flank bars (in males), significantly differ from the values obtained for the other examined species. Moreover, there is a significant correlation between SL and numbers of flank bars (Pearson Correlation $\mathrm{r}=0.455, \mathrm{p}<0.05^{*}$ ).

The otolith is rounded-trapezoid and characterized by a very wide excisura, a me-dium-sized and pointed rostrum, and a quite short antirostrum. The ventral and dorsal rims are slightly curved; the ventral rim may bear small irregular processes; the dorsal rim may show a fine crenulation; the posterior rim is steep (Fig. 3W-Aa).

The flank bars in males (Fig. 2a) are narrow and the interspaces are broader than the bars. The first bar is located above the operculum, while the posteriormost bar is located at the base of the caudal fin; the interspaces are wider at the caudal peduncle than in the anterior body part. Dorsally, the head is gray and the body is dark due to a strong melanophore pigmentation. The ventral body portion does not usually show


Figure 3. Left otoliths (medial view) of Aphanius isfahanensis (A-F), A. farsicus (G-L), A. sophiae (M$\mathbf{Q})$, A. vladykovi ( $\mathbf{R}-\mathbf{V}$ ) and $A$. arakensis ( $\mathbf{W}-\mathbf{A a}$ ). Otolith terminology and taxonomic most informative morphometric distances are indicated in Fig. 3F and include height of antirostrum (a-c), height of rostrum ( $\mathbf{c - e}$ ), length of antirostrum ( $\mathbf{b}-\mathbf{g}$ ), and length of rostrum ( $\mathbf{d}-\mathbf{f}$ ). SEM pictures.
any dark pigmentation. The dorsal, anal and caudal fins have white margins; the first rays of the dorsal fin are dark. The pectoral fins are somewhat yellowish. The pelvic fin is yellowish. Most specimens are characterized by dark blotches at the base of the dorsal and anal fins.

Females (Fig. 2b) are characterized by a grayish pigmentation of the back. The lateral flanks of the body are covered by dark pigmentations; series of blotches are present from the middle of the body to the caudal peduncle. The ventral part of the head and belly are light. The chin and sides of the head are speckled with melanophores. Below the eye there is a line of relatively dark melanophores. All fins are white.

Comparative remarks. Aphanius arakensis is close to the other Iranian Aphanius species in having a similar external morphology but differs by a high number of flank bars, 12-19, commonly, 15-17 (vs. 8-13, commonly, 11-12 in A. vladykovi; 10-16, commonly, 12-13, in A. farsicus; 8-15, commonly, 11-13 in $A$. sophiae; 9-13, commonly, 10-11 in $A$. isfahanensis and 11-16, commonly, 13-14 in A. pluristriatus), otolith morphology and by having 19 molecular apomorphies in the cytochrome $b$ gene. The new species (both males and females) can be further distinguished from $A$. vladykovi by 28-32 lateral line series scales (vs. 33-43), and by less relative width and length of scales, 3.3-4.6 and 3.3-4.5\% SL, respectively (vs. 1.9-3.2 and 1.9-3.3, respectively). It differs from A. sophiae in having 10-13 caudal peduncle scales (vs. 8-11), less gill rakers numbers, 8-10 (vs. 9-12), and by a greater interorbital distance, $0.9-1.2 \%$ head width (vs. $0.8-1.1$ ). The new species differs from $A$. farsicus in having $6-8$ pelvic fin rays (vs. 6-7), and by a smaller eye diameter, $10.7-14.8 \%$ preanal distance (vs. $10.6-17.0$ ). It can be distinguished from $A$. isfahanensis by $8-10$ gill rakers (vs. 10-13), and by a shorter caudal peduncle, 29.0-34.8\% preanal distance (vs. 27.4-38.4). It differs from $A$. pluristriatus in having 10-13 caudal peduncle scales (vs. 8-11), 28-32 lateral line series scales (vs. 24-29) and by a smaller eye diameter, 10.7-14.8\% preanal distance (vs. 12.4-18.1).

Distribution and habitat. The species has been collected from a small natural shallow pond (Fig. 4) in the Namak Lake basin, 5 km south east of the city of Arak (Fig. 1). This pond, which is about $6 \times 4 \mathrm{~m}$ in size, is fed by the drainage of a nearby natural spring. During sampling, the water body was almost stagnant and water temperature was $23^{\circ} \mathrm{C}$. There was no vegetation in the pond, but the surrounding area was covered with Juncus sp. and Typha sp. The bottom of the pond was generally muddy with small gravels. The habitat was in a bad condition due to anthropogenic pollution. Around collection time, the new Aphanius species was the only fish observed living in the pond. In addition, the new species can be found in several springs located in close proximity to the type locality (Fig. 5).

Etymology. The species name refers to the city of Arak, which is located in close proximity to the type locality. Arak is the capital of the Markazi province in northcentral Iran. A proposed common name is Arak tooth-carp. Farsi name is Kapour-e-dandandar-e-Arak.


Figure 4. Natural shallow pond and type locality of Aphanius arakensis sp. n., in the Namak Lake Basin, 5 km SE of Arak city, Iran (see Fig. 1).


Figure 5. Male (above) and female specimens (not preserved) of Aphanius arakensis sp. n., collected from Cheshmeh Nazi (Nazi spring, $33^{\circ} 42^{\prime} 56.8^{\prime \prime} \mathrm{N} 50^{\circ} 04^{\prime} 21.9^{\prime \prime} \mathrm{E}$ ) near type locality, Namak Lake Basin.

Table 3. Summary of diagnostic molecular characters that differentiate Aphanius arakensis sp. n., from other Iranian Aphanius species. Of the 19 molecular apomorphies, 17 are transitions and two are transversions. Numbers above characters indicate the character's position in the complete molecular character matrix.

| Position | 2 | 2 | 2 | 3 | 3 | 4 | 5 | 5 | 6 | 6 | 6 | 6 | 7 | 7 | 8 | 8 | 8 | 8 | 8 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 0 | 7 | 9 | 3 | 9 | 1 | 2 | 4 | 0 | 1 | 2 | 2 | 4 | 9 | 1 | 3 | 6 | 7 | 8 |
|  | 7 | 6 | 1 | 3 | 5 | 1 | 6 | 9 | 6 | 6 | 2 | 4 | 5 | 5 | 1 | 7 | 0 | 5 | 7 |
| A. arakensis | G | T | C | T | G | C | A | G | T | A | A | G | G | T | G | T | T | G | C |
| A. isfahanensis | A | C | T | C | A | G | G | A | A | G | G | A | A | C | A | C | C | A | T |
| A. sophiae | A | C | T | C | A | G | G | A | A | G | G | A | A | C | A | C | C | A | T |
| A. farsicus | A | C | T | C | A | G | G | A | A | G | G | A | A | C | A | C | C | A | A |
| A. vladykovi | A | C | T | C | A | A | G | A | A | G | G | A | A | C | A | C | C | A | A |

## Phylogenetic relationships

The parameters for the maximum likelihood are $\ln (\mathrm{L})=-85.11 .91237$, gamma shape parameter of 1.000 , proportion of invariant sites of 0.097 and parsimony $=1556$. The maximum parsimony phylogeny has a CI of 0.462 and RI of 0.747 . The initial tree for the maximum likelihood analysis was obtained by the BIONJ algorithm. The trees of the maximum likelihood and maximum parsimony phylogenies (Fig. 6) are not significantly different in topology (Templeton test, $\mathrm{P}>0.05$ ). They support the hypothesis that Aphanius arakensis diverged from the clade leading to the present-day A. sophiae and is sister to this species. Moreover, $A$. farsicus is sister to $A$. arakensis $+A$. sophiae; sister to these taxa is $A$. isfahanensis, and sister to all previously mentioned species is $A$. vladykovi. The same topology (Templeton test, $\mathrm{P}>0.05$ ) is observed for the tree of the Neighbor Joining (NJ) distance-based analysis. Table 4 shows the estimation of evolutionary divergence between the sequences of the new species and its relatives.

## Discussion

## Probable reasons for morphological similarities between endemic Aphanius species

Several endemic Aphanius species are known that are soundly circumscribed by genetic differentiation and specific otolith morphology (see below), whereas they differ only weakly (or only in multivariate space) with regard to morphometry and meristics. Examples are $A$. isfahanensis from central Iran, A. sophiae and $A$. farsicus from southern Iran (Hrbek et al. 2006, this study); another example from the Mediterranean area is A. baeticus from Spain (Doadrio et al. 2002). Aphanius arakensis sp. n., from the Namak Lake basin represents yet another example for a species that is difficult to distinguish from its relatives based on external characters (with the exception of the features mentioned above). It is likely that the overall morphological similarity between


Figure 6. Phylogenetic relationships of Aphanius arakensis sp. n., and other endemic species of Aphanius in Iran as indicated by maximum likelihood (based on cytochrome b sequences) and phenetic (based on morphometric characters of fish specimens + J scale indices) analysis. Numbers above nodes represent maximum likelihood bootstrap values based on 2000 replicates. Species and locations correspond to those listed in the Material section.
these taxa are a result of the similar habitats, in which the various endemic Aphanius species are thriving. Thus, common environmental variables may have acted as a stabilizing selection on morphological characters (see also Hrbek et al. 2006). This offers an explanation as to why speciation events in Aphanius have affected genetic characters, rather than morphology, and why rapid genetic diversification can occur with little morphological change in this taxon (see also Adams et al. 2009).

## Probable reasons for otolith differences between endemic Aphanius species

Otolith morphology is known to support the distinctive taxonomic state of several Aphanius species (Reichenbacher et al. 2007, 2009a-b). However, otolith morphology has not been used in previous studies on the endemic Iranian inland Aphanius species. Here we have compared the otoliths of $A$. vladykovi, $A$. isfahanensis, A. farsicus
Table 4. Estimation of Genetic divergence (Kimura 2-parameter model) between the sequences of the Aphanius arakensis sp. n., and other Iranian Aphanius species. $\mathrm{Aa}=$ Aphanius arakensis, $\mathrm{Ai}=A$. isfahanensis, $\mathrm{As}=A$. sophiae, $\mathrm{Af}=A$. farsicus and $\mathrm{Av}=A$. vladykovi.

|  | Aa1 | Aa2 | Aa3 | Af1 | Af2 | Af3 | Af4 | Ai1 | Ai2 | Ai3 | As1 | As2 | As3 | As4 | As5 | As6 | Av1 | Av2 | Av3 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Aa1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Aa2 | 0.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Aa3 | 0.001 | 0.001 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Af1 | 0.057 | 0.057 | 0.056 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Af2 | 0.054 | 0.054 | 0.053 | 0.009 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Af3 | 0.047 | 0.047 | 0.045 | 0.009 | 0.008 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Af4 | 0.047 | 0.047 | 0.045 | 0.011 | 0.007 | 0.001 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Ai1 | 0.119 | 0.119 | 0.118 | 0.116 | 0.115 | 0.113 | 0.114 |  |  |  |  |  |  |  |  |  |  |  |  |
| Ai2 | 0.098 | 0.098 | 0.096 | 0.092 | 0.093 | 0.090 | 0.092 | 0.025 |  |  |  |  |  |  |  |  |  |  |  |
| Ai3 | 0.113 | 0.113 | 0.111 | 0.111 | 0.109 | 0.105 | 0.106 | 0.012 | 0.019 |  |  |  |  |  |  |  |  |  |  |
| As1 | 0.041 | 0.041 | 0.040 | 0.068 | 0.066 | 0.058 | 0.060 | 0.118 | 0.097 | 0.112 |  |  |  |  |  |  |  |  |  |
| As2 | 0.029 | 0.029 | 0.027 | 0.053 | 0.051 | 0.042 | 0.044 | 0.105 | 0.084 | 0.099 | 0.015 |  |  |  |  |  |  |  |  |
| As3 | 0.036 | 0.036 | 0.034 | 0.054 | 0.051 | 0.050 | 0.051 | 0.106 | 0.085 | 0.100 | 0.018 | 0.007 |  |  |  |  |  |  |  |
| As4 | 0.029 | 0.029 | 0.027 | 0.053 | 0.051 | 0.042 | 0.044 | 0.105 | 0.084 | 0.099 | 0.015 | 0.000 | 0.007 |  |  |  |  |  |  |
| As5 | 0.030 | 0.030 | 0.029 | 0.051 | 0.053 | 0.044 | 0.045 | 0.107 | 0.084 | 0.100 | 0.016 | 0.001 | 0.008 | 0.001 |  |  |  |  |  |
| As6 | 0.033 | 0.033 | 0.031 | 0.054 | 0.052 | 0.047 | 0.048 | 0.108 | 0.087 | 0.102 | 0.018 | 0.004 | 0.008 | 0.004 | 0.005 |  |  |  |  |
| Av1 | 0.095 | 0.095 | 0.093 | 0.092 | 0.090 | 0.084 | 0.086 | 0.121 | 0.106 | 0.118 | 0.096 | 0.083 | 0.088 | 0.083 | 0.083 | 0.081 |  |  |  |
| Av2 | 0.082 | 0.082 | 0.081 | 0.077 | 0.073 | 0.075 | 0.076 | 0.115 | 0.098 | 0.112 | 0.086 | 0.070 | 0.073 | 0.070 | 0.072 | 0.075 | 0.027 |  |  |
| Av3 | 0.107 | 0.107 | 0.105 | 0.102 | 0.103 | 0.098 | 0.100 | 0.097 | 0.111 | 0.094 | 0.109 | 0.094 | 0.097 | 0.094 | 0.096 | 0.099 | 0.034 | 0.029 |  |



Figure 7. Phylogenetic relationships of Aphanius arakensis sp. n., and other endemic species of Aphanius in Iran as indicated by maximum parsimony (based on cytochrome b sequences) analysis. The maximum parsimony phylogeny has a CI of 0.462 and RI of 0.747 . Numbers above nodes represent maximum parsimony bootstrap values based on 2000 replicates.
and $A$. sophiae (Fig. 3) to show that these species are clearly different with regard to otolith morphology. Also $A$. arakensis shows clear divergence of its otolith morphology in comparison to the other inland Aphanius species, in particular with regard to the weakly pronounced antirostrum (Fig. 3W-Aa).

Notably, the otoliths of $A$. vladykovi are most distinctive in comparison to those of the other studied species as they are characterized by a long ventral part, angular overall shape and long rostrum (Fig. 3R-V). This uniqueness of the $A$. vladykovi otoliths corresponds well to our and previous phylogenetic analyses, which have established $A$. vladykovi as being sister to all other Iranian inland species that diverged approximately 10 Ma ago (Hrbek et al. 2003). As a result, Aphanius likely has a higher rate of divergence in otolith morphology than in overall morphology. This difference in divergence rate may be related to the function of the otoliths as parts of the inner ear. In general, otoliths provide a mechanism for measuring motion and position of the head relative to gravity (Manley et al. 2004). However, it is quite important for a fish to know from where a sound is coming, so as to be able to distinguish between different sounds and pick out the biologically most relevant sounds (Popper et al. 2005). In addition, differences in otolith morphology are related to the balance and orientation of a fish (Popper et al. 2005). This means that differences in otolith morphology can reflect changes in intraspecific communication and behavior in fishes, that may have acted as evolutionary pressures.

## Role of coloration pattern (flank bar numbers) in Aphanius diversification

Coloration and flank bar numbers are significant characters for the identification of Aphanius species, in particular for the identification of male individuals. Among the allopatric Iranian Aphanius species, males of $A$. arakensis have the largest number of flank bars, and flank bars are non-overlapping, whereas the number of flank bars is lowest in A. sophiae. Also the flank bars of the central Anatolian Aphanius species vary in thickness and number between species (Hrbek et al. 2002). However, the mechanisms underlying male flank bar variation have not been studied. We hypothesize that flank bar patterns play an important role in sexual selection, and thus represent important factors in the evolutionary history and speciation of Aphanius.

Sexual selection has long been believed to promote species divergence among groups of animals (see Kraaijeveld et al. 2010 for a review). Sexual selection may facilitate speciation because it can cause rapid evolutionary diversification of male mating signals and female preferences (Boughman 2001). Divergence in these traits may then contribute to reproductive isolation.

Several studies indicate that fishes can adapt to variation in underwater light environments by changing their colour, most likely as a result of a more effective intraspecific communication (Boughman 2001, 2002; Fuller 2002; Seehausen et al. 2008). Adding support to this interpretation is provided by studies on cichlids from the Victoria Lake (Seehausen et al. 2008) and African elephant fishes (Leal and Losos 2010). These studies indicate that variation in male nuptial coloration due to specific light conditions in different environments can result in ecological, phenotypic, genetic and behavioral differentiation. Additionally, color contrast with the visual background was found to be more important for effective intraspecific communication
than color brightness (Fuller 2002). Thus, our conclusion is that the specific male flank bar patterns in different Aphanius species may have evolved as a response to different light regimes prevalent in respective habitats for increasing contrast and optimizing intraspecific communication. It can therefore be suggested that sensory-driven speciation might have played a prominent role in Aphanius speciation.

## Conclusion

The noticeable features of the present-day diversity of the endemic Aphanius species in Iran include high genetic divergence and clear differences in otolith morphology, but only weak differences in general external morphology, morphometry and meristics. These patterns are probably caused by different rates of evolution in the mentioned characters that may be linked to the similarity of the individual environments, intraspecies communication, and vicariance events. It is likely that additional Aphanius species are present in remote areas of Iran, especially in the Zagros and Alburz Mountains.

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# An extraordinary new family of spiders from caves in the Pacific Northwest (Araneae, Trogloraptoridae, new family) 

Charles E. Griswold ${ }^{1,2,3, \dagger}$, Tracy Audisio ${ }^{1,2,4, \ddagger}$, Joel M. Ledford ${ }^{1,3, \S}$<br>I Arachnology Lab, California Academy of Sciences 2 Department of Biological Sciences, San Francisco State University 3 Environmental Science, Policy and Management, University of California, Berkeley 4 Summer Systematics Institute, California Academy of Sciences<br>$\dagger$ urn:lsid:zoobank.org:author:0676B242-E441-4715-BF20-1237BC953B62<br>$\ddagger$ urn:lsid:zoobank.org:author:EE3A6634-D76D-4F1E-B117-8D6BB1285223<br>§ urn:lsid:zoobank.org:author:F55EE588-CC9C-4348-ADOF-30F8336DEDA3<br>Corresponding author: Charles E. Griswold (cgriswold@calacademy.org)

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#### Abstract

The new spider genus and species Trogloraptor marchingtoni Griswold, Audisio \& Ledford is described as the type of the new family Trogloraptoridae. The oblique membranous division of the basal segment of the anterior lateral spinnerets of Trogloraptor suggests that this haplogyne family is the sister group of the other Dysderoidea (Dysderidae, Oonopidae, Orsolobidae and Segestriidae). Trogloraptor is known only from caves and old growth forest understory in the Klamath-Siskiyou region of Oregon and California.


## Keywords

Haplogynae, Caves, Pacific Northwest

## Introduction

The spider fauna of North America is a rich one, with at least 68 families including 569 genera and comprising more than 3700 species (Ubick et al. 2005). In the last generation significant progress has been made at understanding this fauna. For example,
continent-wide taxonomic treatments of major families have appeared, e.g., Araneidae (Levi 1968 to present), Gnaphosidae (Platnick 1975 and subsequent) and Theridiidae (Levi 1953 and subsequent), the largest genera of jumping spiders (Salticidae) have been revised, i.e., Habronattus (Griswold, 1987), Pelegrina (Maddison, 1996) and Phidippus (Edwards, 2004) and the Canadian government has produced a series of guides to Canadian spiders, e.g., Dondale and Redner (1982). Perhaps most important was Vince Roth's key to families and genera (Roth 1982), which has been updated as the comprehensive and profusely illustrated Spiders of North America: an identification manual (Ubick et al. 2005). This makes it relatively easy to identify to family and even to genus any spider from America north of Mexico. Nevertheless, there are still surprises in store in this well known region, especially in remote or inaccessible places, such as caves. The troglofauna of the Pacific Northwest is poorly surveyed and several arachnid groups are known to have multiple undescribed species (Ledford et al. in prep.). In this paper we describe a remarkable haplogyne spider that fits into no known family, living or extinct (Jocqué and Dippenaar-Schoeman 2006; Penney and Selden 2011). Trogloraptor marchingtoni gen. et sp. n., type of the new family Trogloraptoridae, is described from caves in the Pacific Northwest, and a diagnostic table (Table 1) and character discussion are presented to distinguish it from all other spider families.

## Materials and methods

Species descriptions refer to a single adult individual for each sex, which is identified as a type or by the locality at which it was collected. All measurements are in millimeters and quantify the size of a structure at its widest or longest point. A section reporting the variation in the most conspicuous and variable features follows each description and represents multiple individuals ( n ), encompassing the full range in overall size.

Prior to examination with a Leo 1450 VP Scanning Electron Microscope, all structures were cleaned with a fine brush and critical point dried. Spinneret preparations followed the methods of Griswold et al. (2005) consisting of a brief cleaning in an ultrasonicator and a gentle squeeze of the abdomen using forceps locked in place with a paperclip followed by overnight immersion in $100 \%$ ethanol in order to extend and separate the spinnerets. Large structures were examined using a Leica MZ 12.5 or MZ 16 stereomicroscope. Vulvae were carefully excised and placed in a pancreatin solution for 24-48 hours to digest extraneous tissue (Álvarez-Padilla and Hormiga 2007) then placed in water and manually cleaned. Light micrographs were prepared using a Nikon DXM1200 digital camera attached to a Leica MZ 16 stereomicroscope or a Leica DM 4000 compound microscope; multiple, stacked images were montaged using the program Helicon Focus ${ }^{\ominus}$. The male pedipalp was checked for haematodochal expansion by placing it into boiling water for 2-3 minutes in a vial of hot $92 \%$ lactic acid solution (Sigma Aldrich, St Louis MO, USA), after which the pedipalp was transferred to distilled water (Ledford et al. 2011). The internal anatomy of the pedipalpal bulb and course of the reservoir were examined by immersing it in a solution of methyl salicylate
Table I. Diagnostic characters for Haplogynae families. Italics represents diagnosis from Trogloraptoridae.

| Family | AME | PME distance | Cheliceral bases | Cheliceral lamina | Posterior receptaculum | 3rd <br> Entapophyses | Posterior spiracles | Posterior spiracle(s) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Caponiidae | present | separate | fused | present | absent | separate, short | two | advanced |
| Diguetidae | absent | contiguous | fused | present | absent | fused | one | posterior |
| Drymusidae | absent | contiguous | fused | present | absent | fused | one | posterior |
| Dysderidae | absent | contiguous | free | absent | present | separate, short | two | advanced |
| Filistatidae | present | contiguous | fused | present | absent | separate, long | two | posterior |
| Leptonetidae | absent | contiguous | free | absent | absent | separate, long | one | posterior |
| Ochyroceratidae | absent | contiguous | free | present | absent | separate, short | one | posterior |
| Oonopidae | absent | contiguous | free | absent | present | separate, short | two | advanced |
| Orsolobidae | absent | contiguous | free | absent | present | separate, short | two | advanced |
| Periegopidae | absent | contiguous | fused | present | absent | fused | one | posterior |
| Pholcidae | present | separate | fused | present | absent | absent | one | posterior |
| Plectreuridae | present | separate | fused | present | absent | fused | one | posterior |
| Scytodidae | absent | contiguous | fused | present | absent | fused | one | posterior |
| Segestriidae | absent | contiguous | free | absent | present | separate, short | two | advanced |
| Sicariidae | absent | contiguous | fused | present | absent | fused | one | posterior |
| Telemidae | absent | contiguous | free | absent | absent | absent | two | posterior |
| Tetrablemmidae | absent | contig./sep. | free | present | absent | fused | one | posterior |
| Trogloraptoridae | absent | separate | free | absent | absent | separate, long | one | posterior |


| Family | Emerit's <br> glands | Tarsal claw <br> number | Serrula tooth <br> rows | Labium-sternum <br> junction | ALS basal <br> segment | PLs AC gland <br> spigots | CY gland spigots | Colulus |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Caponiidae | absent | three | one | fused | entire | dispersed | absent | small |
| Diguetidae | absent | three | one | fused | entire | dispersed | absent | small |
| Drymusidae | absent | three | one | fused | entire | dispersed | absent | small |
| Dysderidae | absent | three | one | free | divided | dispersed | absent | small |
| Filistatidae | absent | three | one | fused | entire | dispersed | absent | absent |
| Leptonetidae | present | three | one | fused | entire | in line | present | small |
| Ochyroceratidae | absent | three | one | fused | entire | in line | absent | large |
| Oonopidae | prs; abs | two | one | free | divided | dispersed | absent | small |
| Orsolobidae | absent | two | one | free | divided | dispersed | absent | small |
| Periegopidae | absent | three | one | free | entire | dispersed | absent | small |
| Pholcidae | absent | three | one | fused | entire | dispersed | absent | small |
| Plectreuridae | absent | three | one | free | entire | dispersed | absent | small |
| Scytodidae | absent | three | one | fused | entire | dispersed | absent | large |
| Segestriidae | absent | three | one | free | divided | dispersed | absent | small |
| Sicariidae | absent | two | one; abs | fused | entire | dispersed | absent | large; abs |
| Telemidae | present | three | one | fused | entire | inline | present | large |
| Tetrablemmidae | absent | three | one | free | entire | dispersed | absent | small |
| Trogloraptoridae | absent | three | multiple | fused | divided | dispersed | absent | small |

(synthetic oil of wintergreen), from Mallinckrodt Baker Chemicals, Philipsburg NJ, USA (Miller et al. 2009). Abbreviations used in the text and figures are as follows: AC, aciniform gland spigots; ALE, anterior lateral eyes; ALS, anterior lateral spinnerets; AME, anterior median eyes; AT, atrium; BM, Brent McGregor; CG, Charles Griswold; ITC, inferior tarsal claw; JL, Joel Ledford; MAP, major ampullate gland spigot(s); mAP, minor ampullate gland spigot(s); Nu , nubbin (an aborted spigot); OAL, ocular area length; PER, posterior eye row; PI, piriform gland spigots; PLE, posterior lateral eyes; PLS, posterior lateral spinnerets; PME, posterior median eyes; PMS, posterior median spinnerets; RC, receptaculum; RD, Ron Davis; STC, superior tarsal claw(s). All specimens are deposited in the California Academy of Sciences (CAS).

## Phylogenetic placement

Trogloraptoridae have simple, haplogyne genitalia, with a single opening of the female vulva for fertilization and oviposition. This family differs from most other haplogyne clades. The Palpimanoidea (Archaeidae, Huttoniidae, Mecysmaucheniidae, Palpimanidae and Stenochilidae) have a foramen around the cheliceral bases, two protrusions posterior to the labral tongue, cheliceral peg teeth and a cheliceral gland mound (Wood et al. in press), all lacking in trogloraptorids. Austrochiloidea (Austrochilidae and Gradungulidae) have a clypeal hood, notched trichobothrial bases, and cylindrical gland spigots (Griswold et al. 2005), again all lacking in trogloraptorids. In spite of striking similarities in the raptorial and asymmetrical claws between gradungulids and trogloraptorids, these families are only distantly related. Trogloraptorids are not Entelegynae with secondarily simplified genitalia: the primitive tapetum in the indirect eyes (Figs 9, 11) and absence of tartipores from the spinnerets (Figs 69-74) rule this out (Griswold et al. 2005). The piriform pedipalpal bulb (Figs 9,51-58) of the male, with tegulum and subtegulum fused, is a synapomorphy placing Trogloraptor in the clade Haplogynae. This clade contains, in addition to the new family Trogloraptoridae, 17 other families: Caponiidae, Diguetidae, Drymusidae, Dysderidae, Filistatidae, Leptonetidae, Ochyroceratidae, Oonopidae, Orsolobidae, Periegopidae, Pholcidae, Plectreuridae, Scytodidae, Segestriidae, Sicariidae, Telemidae and Tetrablemmidae (Platnick et al. 1991; Ramírez 2000). Haplogyne families are among the most clearly defined in the Araneae and well characterized by features of the chelicerae, spinning organs and posterior respiratory system. Trogloraptoridae are distinguished from Caponiidae, Diguetidae, Drymusidae, Filistatidae, Periegopidae, Pholcidae, Plectreuridae, Scytodidae and Sicariidae in having the chelicerae free at the base (Figs 9, 19, 23) (not fused at the base), from Dysderidae, Oonopidae, Orsolobidae, Segestriidae and Telemidae in having a single posterior spiracle (Figs 12, 60, 83) (not two posterior spiracles), from most Leptonetidae and Ochyroceratidae in having scattered AC gland spigots (Figs 73-80) (not AC gland spigots in rows on the PMS and/ or PLS) and having the PME widely separated (Figs 9, 11) (not contiguous), from all Leptonetidae in having coxa-trochanter leg autospasy (not at the patella-tibia) and from Tetrablemmidae (as well as Diguetidae, Drymusidae, Periegopidae, Plectreuridae, Scyto-
didae and Sicariidae) in having long, separate $3^{\text {rd }}$ abdominal entapophyses (Figs 60, 63, 64) (not fused entapophyses). For simplicity we depict 16 diagnostic characters across the 18 families of Haplogynae in Table 1. Another striking feature is the serrula with multiple tooth rows, otherwise found in Araneomorphae only in Hypochilidae. In many ways Trogloraptoridae are a collection of primitive character states. A clue to this family's phylogenetic affinity comes from the basal segment of the ALS: the segment is crossed diagonally by a line of membranous cuticle, contrasting with the surrounding sclerotization (Fig. 68). This morphology was first reported by Simon (1893: 310) in Dysderidae, and is a probable synapomorphy for the Dysderoidea (Dysderidae, Oonopidae, Orsolobidae and Segestriidae) (Fig. 65). It is lacking in other Haplogynae, e.g., Figures 66, 67. This peculiar spinneret morphology in Trogloraptoridae suggests that this family is allied to the Dysderoidea. If so, the family Trogloraptoridae is a primitive member of Dysderoidea, lacking the paired, anteriorly advanced posterior tracheal spiracles and posterior receptaculum of the vulva that are synapomorphies uniting the four remaining families, Dysderidae, Oonopidae, Orsolobidae and Segestriidae.

## Taxonomy

## Trogloraptoridae Griswold, Audisio \& Ledford, fam. n.

 urn:lsid:zoobank.org:act:0B46EFED-0BE4-4618-B14C-0EFFAA7CA4EATypes. Trogloraptor marchingtoni Griswold, Audisio and Ledford, here designated.
Diagnosis. Ecribellate Haplogynae lacking AME, with ALE and PLE contiguous but PME separated (Figs 9, 11), chelicerae free at base and distally not forming a chela with fang (Figs 9, 10, 21, 24), Emerit's glands absent from patellae and tibiae (Fig. 37), posterior respiratory system with broad spiracle closer to spinnerets than to epigastric furrow (Figs 12, 83), with paired, 2-branched lateral tracheal tubes and long, separate median entapophyses (Figs 60, 63), ALS basal article crossed by a diagonal membranous area (Fig. 68), and with all leg tarsi subsegmented and raptorial (Figs 13, 14, 29-32, 38, 44).

Synapomorphies. The extraordinary, subsegmented raptorial leg tarsi are unique among spiders and clearly autapomorphic for the family.

## Trogloraptor Griswold, Audisio \& Ledford, gen. n.

 urn:lsid:zoobank.org:act:25F85266-612A-42BC-B2F9-72D3B5E0F7DC http://species-id.net/wiki/TrogloraptorType species. Trogloraptor marchingtoni new species, here designated.
Etymology. The generic name refers to the cave habitat and raptorial tarsi.
Diagnosis. By the characters of the family.
Synapomorphies. As for the family.

Description. Cephalothorax with carapace pear-shaped, narrowed anteriorly, pars cephalica faintly distinguished from pars thoracica, fovea indistinct (Figs 11, 16); six eyes, AME absent, ALE and PLE contiguous, PME separated from lateral eyes by their diameter, separated from each other by more than twice their diameter, shiny tapeta fill entire eyecup, of "primitive" type (Homann 1971) (Figs 9, 11); clypeus high, more than six times PME diameter, sloping anteriorly, ventral margin straight (Fig. 9); chelicerae free at base, without a boss (Figs 9, 22), with weak mesal lamellar ridge for basal $2 / 3$ (Fig. 23), fang furrow with one large distal prolateral tooth and two promarginal and two retromarginal small proximal teeth; promargin with more than 30 elongate setae and setae on both margins at fang base (Figs 20, 21, 24), cheliceral gland opens as sparse pores near position of fang tip (Figs 20); fang without serrations along inner margin, apex longer than base, poison gland pore subapical, retrolateral (Figs 23, 24); no apparent chilum beneath clypeus but with small anterior sclerite between cheliceral bases, intercheliceral sclerite a narrow rectangle (Fig. 9); labrum elongate, with numerous plumose setae from base to middle, labral tongue free, longitudinally wrinkled apically, with minute, bristle-like setae distad of tongue apex (Fig. 27); pedipalpal coxa narrow, pointed apically, with membranous cuticle at apex and retroapical serrula (Fig. 10), serrula teeth in two rows (Figs 25, 26), with dorsal maxillary gland opening mesally near labrum (Figs 27, 28); labium narrow, sides converging, with weak basal notches, fused to sternum (Fig. 10); sternum heart-shaped, laterally undulate, anterior margin rounded on each side of labium, laterally with narrow lobes opposite coxae and rounded lobes between coxae, without free sclerites, posteriorly narrowly rounded between coxae IV (Fig. 18); coxae cylindrical, without retrocoxal hymen, trochanters shorter than coxae, apices straight, without notch (Fig. 18); narrow, slightly curved supracoxal sclerites above each leg coxa; leg formula 1243, legs elongate, femur I 1.69-2.30 times carapace length (Figs $1-8,15,17$ ), sparsely covered with plumose setae, cuticle smooth or with fine fingerprint pattern (Figs 45-50), autospasy at coxa/trochanter joint, pairs of small sclerites visible in intersegmental membranes between coxae-trochanters and femora-patellae, metatarsi III and IV of female with ventrolateral patch of curved, spinose setae (Figs 29, 33), densest proventrally, Emerit's glands absent from patellae (Fig. 37) and tibiae, legs with few spines except beneath tarsi I-III, pedipalp with dorsoapical spine on patella and median prolateral on tibia, female pedipalpal tarsus with three prolateral and one retrolateral spines; tarsal trichobothria absent, with only a single, subapical trichobothrium on metatarsi, 1-3 dorsal trichobothria on leg tibiae, more on pedipalpal tibia, bothrium with proximal hood (Fig. 50) or a smooth, entire ring, narrower apically (Fig. 49), trichome plumose, slightly narrowed basally (Fig. 49); tarsal organ near apex of pedipalp, at mid point of leg tarsi proximad of second membranous subsegmentation (Fig. 45), exposed, round, nearly flat, with central depressed circle or 1-2 raised sensillae (Figs 46-48); leg tarsi raptorial (Figs 5, 6, 13, 14, 29-32, 34), with flexible subsegmentations (Figs 13, 14, 30) near base and subapically in female tarsi I-IV, male I-III, subapical only in male tarsus IV, tarsi I-III with paired stout spines ventrally, one pair proximad of and three pair distad of basal subsegmentation (Figs 13, 40, 43, 44), tarsus IV lacks such spines (Figs 14, 30); leg tarsi with three claws, STC I-III slightly asymmetrical, proclaw longer (Figs 13, 43), STC teeth uniseriate, proclaws I-III with 8-9 teeth and fine basal comb, retroclaw with 15-25 fine
teeth, STC IV sexually dimorphic, female claws equal (Fig. 44), male asymmetrical, retroclaw with 22 teeth, proclaw short, palmate, with one large and fan of 9 smaller teeth (Figs 14, 39), ITC long, curved, with distal and proximal teeth, tarsus without serrate accessory setae, claw tufts or scopulae (Figs 35, 36, 40-43); female pedipalp with long, smooth claw (Figs 35, 36). Abdomen oval, unsclerotized except at book lung openings, sparsely covered with setae (Figs 12, 15-18); pedicel with ventral sclerite contiguous to sternum, dorsum with lorum divided anteriorly (Fig. 16); male lacks epiandrous spigots (Fig. 82); anterior respiratory system booklungs, posterior respiratory system with broad spiracle closer to spinnerets than to epigastric furrow (Figs 12, 83), with paired, 2-branched lateral tracheal tubes and long, separate median entapophyses (Figs 60, 63), entapophyses tips frayed as muscle attachments (Fig. 64); colulus a large, oval sclerotized lobe, covered with hairs (Figs 75, 81); ALS with three segments (Fig. 69), basal segment divided obliquely by membranous cuticle (Fig. 68), with about 30 PI gland spigots, each with convex base and a narrow tapering shaft, shaft origin slightly sunken into base and encircled by a cuticular ridge (Fig. 71), female mesally with anterior large and posterior small MAP gland spigots (Figs $69,72,84$ ); male resembles female except posterior MAP gland spigot replaced by a small nubbin (Fig. 70); PMS of female with two spigots with squat bases and narrow shafts (Figs 77,78 ), male retains only the posterior (Fig. 80), suggesting that this is an AC gland spigot and the anterior is a mAP gland spigot, cuticle on mesal surface of PMS wrinkled (Fig. 77); PLS of female (Figs 73, 74) and male (Fig. 86) with two spigots with squat bases and narrow shafts, these probably AC gland spigots; female genitalia haplogyne, anterior edge of epigastric furrow sclerotized, vulva internally (Figs 59,61) with median atrium and paired, lateral receptacula with sclerotized stalks and membranous apical bulbs (Fig. 62), apical bulbs may serve as muscle attachments; male pedipalp femur to tarsus lacking apophyses, cymbium narrow, without trichobothria or chemosensory scopulae, extending far beyond base of bulb (Figs 9, 15, 17, 51, 52, 57); male pedipalpal bulb piriform, swollen, lacking processes, embolus long, slender, recurved apically (Figs 51-58), spermpore subapical (Fig. 56), reservoir broad, making $11 / 2$ spiral within bulb (Figs 57, 58), basal haematodocha does not expand but bulb orientation twists slightly.

Composition. One species described, probably another known only from juveniles.
Distribution. Known only from caves and old growth forest understory in the Klamath-Siskiyou region of Oregon and California.

## Trogloraptor marchingtoni sp. n.

urn:lsid:zoobank.org:act:DD0946CA-9479-4DC5-BCA2-384B6B82599E
http://species-id.net/wiki/Trogloraptor_marchingtoni
Figures 1-64, 68-86

Types. Holotype male from M2 Cave, 15.7 km SSW Grants Pass, Josephine Co., Oregon, USA, collected 29 July 2010 by R. S. Davis and D. S. Snyder, CASENT9040013, and paratype female from No Name Cave, Josephine Co., Oregon, 17.8 km SSW Grants Pass, collected 16 Sept. 2010 by N. Marchington, CASENT9040065, deposited in CAS.


Figures I-8. Habitus of live Trogloraptor marchingtoni. I-4 female in captivity (JL) $\mathbf{5}$ female in Lake Cave (CG) $\mathbf{6}$ female in M2 Cave (RD) 7, $\mathbf{8}$ female in No Name Cave (BM).


Figures 9-14. Habitus and tarsi of male Trogloraptor marchingtoni (CASENT9040013) from M2 Cave. 9 front $\mathbf{I} \mathbf{0}$ mouthparts, ventral view II carapace, dorsal view $\mathbf{1 2}$ abdomen, ventral view, arrow to tracheal spiracle $\mathbf{1 3}$ tarsus I, prolateral; and $\mathbf{1 4}$ tarsus IV, prolateral.

Etymology. The specific name is a patronym in honor of Neil Marchington, cave biologist, Advisory Board member of the Western Cave Conservatory, Conservation Chair, Western Region, National Speleological Society and Deschutes County Deputy Sheriff, in gratitude for his help and kindness.

Diagnosis. By the characters of the genus.
Male (Holotype). Total length 9.70. Markings as in Figs 9-12, 15-18, cephalothorax, legs and pedipalps yellow-brown, unmarked except for dark brown v-mark posteriorly on pars cephalica, clypeus and chelicerae orange brown, abdomen purple brown with faint light chevrons posteriorly on dorsum. Carapace 4.50 long, 3.10 wide; clypeus 1.33 high; ocular area 0.45 long, 1.30 wide; ratio of eyes ALE:PME:PLE,


Figures 15-18. Habitus of male Trogloraptor marchingtoni (CASENT9040013) from M2 Cave. 15, 16 dorsal views 17, 18 ventral views; note pedicel in Figure 16.
1.08:1.00:1.08; diameter of PME 0.18; chelicerae 2.38 long; sternum 1.75 long, 1.88 wide; labium 1.08 long, 0.70 wide; pedipalpal coxa 1.50 long, 0.45 wide; leg measurements (Femur + Patella + Tibia + Metatarsus + Tarsus $=[$ Total $]):$ I: $8.25+1.40+9.25$ $+9.00+1.45=[29.35]$; II: $7.75+1.35+8.05+8.00+1.40=[26.55]$; III: $6.40+$ $1.40+6.25+5.70+1.60=[21.35] ;$ IV: $7.15+1.40+6.50+6.35+1.50=[22.90]$; pedipalp: $1.55+0.55+1.70+2.40=[6.20]$. Pedipalp as in Figs 9, 16, 51-58.

Variation ( $\mathbf{N}=\mathbf{2}$ ): Total length 6.90—9.70; carapace length 1.19--1.45 times width, height $0.33-0.35$ times width; PER width $2.89-3.00$ times OAL; clypeal height 6.43-7.00 times PME diameter; clypeal height $1.74-2.11$ times cheliceral length; sternum length $0.92-0.93$ times width, labium length $1.54-1.55$ times width, pedipalpal coxa length $2.81-3.33$ times width; femur I length $1.83-2.30$ times carapace length; metatarsus I length $2.03-2.06$ times carapace length.


Figures 19-24. Scanning electron micrographs of the right chelicera of female Trogloraptor marchingtoni (CASENT9040051) from M2 Cave. 19 mesal view 20 mesal view, arrow to cheliceral gland openings $\mathbf{2 I}$ retrolateral view $\mathbf{2 2}$ ectal view $\mathbf{2 3}$ prolateral view, arrow to weak laminar ridge; and $\mathbf{2 4}$ prolateral view, close up of fang and opening of poison gland.

Female (paratype): Total length 9.40. Markings as in male (Figs 1-8). Carapace 3.90 long, 2.90 wide; clypeus 0.93 high; ocular area 0.38 long, 1.28 wide; ratio of eyes ALE:PME:PLE, 1.00:1.00:1.07, diameter of PME 0.15 ; chelicerae 2.25 long; sternum 1.75 long, 1.88 wide; labium 0.95 long, 0.65 wide; pedipalpal coxa 1.38 long, 0.50 wide. Leg measurements (Femur + Patella + Tibia + Metatarsus + Tarsus $=[$ Total $]$ ): I: $7.15+1.25+6.85+7.25+1.35=[23.85]$; II: $6.75+1.30+6.85+6.95+1.40=$ [23.25]; III: $5.25+1.25+5.10+4.70+1.15=[17.50]$; IV: $5.85+1.25+5.60+5.15$ $+1.20=$ [19.05]; pedipalp: $1.50+0.50+1.05+2.05=$ [5.10]. Genital region weakly sclerotized externally, vulva with median translucent atrium and sclerotized lateral receptaculae, receptacular apex membranous (Figs 59, 61, 62).


Figures 25-28. Scanning electron micrographs of the endite of a female Trogloraptor marchingtoni (CASENT9040051) from M2 Cave. $\mathbf{2 5}$ serrula $\mathbf{2 6}$ serrula, close up, note multiple tooth rows $\mathbf{2 7}$ labrum and left endite, dorsal view, arrow to maxillary gland opening $\mathbf{2 8}$ maxillary gland opening, close up.

Variation ( $\mathbf{N}=\mathbf{3}$ ): Total length 8.27-9.60; carapace length $1.30-1.40$ times width, height $0.34-0.39$ times width; PER width $2.71-3.40$ times OAL; clypeal height 6.17-6.91 times PME diameter, 2.05-2.43 times cheliceral length; sternum length $0.93-0.96$ times width; labium length $1.28-1.54$ times width; pedipalpal coxa length $2.75-3.67$ times width; femur I length $1.69-1.83$ times carapace length; metatarsus I length $1.72-1.78$ times carapace length.

Natural history. This species has been collected in the dark zone of caves, hanging beneath a few strands of silk that are attached to the cave roof (Figs 5-8). Boulders and rotting logs were searched near the entrance to M2 Cave without finding any Trogloraptor. Nothing has been observed of its predatory or mating behavior. Living specimens were reared in climate controlled conditions and constructed a loose tangle of web from which they hung beneath. Multiple attempts to feed the specimens a variety of prey items failed, which may indicate a preference for specific prey.

Distribution. Caves in southwestern Oregon.
Additional material examined (all CAS). USA: OREGON: Josephine Co., No Name Cave, 17.8 km SSW Grants Pass, 16 Sept. 2010, N. Marchington, 1 , CASENT9040051, $1 \widehat{J}^{\top}$, CASENT9040066, same data except 13 July 2011, 1 , CASENT9039440; M2 Cave, 15.7 km SSW Grants Pass, 31 July 2010, Geo Graening, R. S. Davis and D. S. Snyder, 1 penultimate , CASENT9040012, same data except 13 July 2011, N. Marchington, T. Audisio, C. Griswold, J. Ledford, D. Ubick,


Figures 29-36. Right appendages of female Trogloraptor marchingtoni (CASENT9040051) from No Name Cave. 29 metatarsus and tarsus of leg III, prolateral view, arrow to ventrolateral patch of curved, spinose setae $\mathbf{3 0}$ tarsus of leg IV, prolateral view, arrows to membranous regions $\mathbf{3 I}$, $\mathbf{3 2}$ tarsus of leg IV, prolateral view $\mathbf{3 3}$ curved setae on metatarsus of leg III, prolateral view $\mathbf{3 4}$ tarsus of leg IV, dorsal view 35 pedipalp, prolateral view; and $\mathbf{3 6}$ tarsal claw of pedipalp.


Figures 37-44. Scanning electron micrographs of legs of Trogloraptor marchingtoni from No Name Cave. $\mathbf{3 7}$ left patella IV, dorsal view $\mathbf{3 8}$ left tarsus IV, retrolateral view $\mathbf{3 9}$ left tarsus IV, prolateral view 40 right tarsus III, retrolateral view 41 right tarsus IV, retrolateral view $\mathbf{4 2}$ left tarsus IV, retroventral view 43 right tarsus III, ventral view, arrows to spines 44 right tarsus IV, apical view. Figures 37-39, 42 (male, CASENT9040066) 40, 41, 43, 44 (female, CASENT9040051).


Figures 45-50. Scanning electron micrographs of sensory organs of Trogloraptor marchingtoni. $\mathbf{4 5}$ right tarsus I, arrow to tarsal organ $\mathbf{4 6}$ tarsus IV, arrow to tarsal organ 47 right tarsus I, tarsal organ 48 tarsal organ on palp $\mathbf{4 9}$ trichobothrium on tibia of R leg III, prolateral view $\mathbf{5 0}$ trichobothrial base on metatarsus of right leg III. Figures 45, 47-49 (female, CASENT9040051) 46 (male, CASENT9040066) 50 (female, CASENT9040041).
H. Wood and F. Álvarez-Padilla, 3 juveniles, CASENT9047599; Lake Cave near No Name Cave, 9.05 km S Wilderville, 13 July 2011, N. Marchington, T. Audisio, C. Griswold, J. Ledford, D. Ubick, H. Wood and F. Álvarez-Padilla, $1 \widehat{h}^{\text {§ }}$ (molted to maturity in captivity) CASENT9047600; Chapman Cave, 12 July 2011, N. Marchington, 2 juveniles, CASENT9039436.

Note. A juvenile Trogloraptor specimen has been collected under debris in old growth redwood forest in far northwest California. The markings of this juvenile differ from the cave species, T. marchingtoni. The northwest California specimen has dusky markings laterally on leg femora, a dusky Y marking extending back from the PLE to the posterior


Figures 5I-58. Male pedipalp of Trogloraptor marchingtoni: 5I-56 Scanning electron micrographs of right pedipalp 51 tarsus and bulb, apical view $\mathbf{5 2}$ tarsus and bulb, dorsal view $\mathbf{5 3}$ bulb, dorsal view 54 embolus, dorsal view $\mathbf{5 5}$ bulb, ventral view $\mathbf{5 6}$ embolus, ventral view, arrow to sperm pore $\mathbf{5 7}, 58 \mathrm{Au}$ tomontage of left pedipalp $\mathbf{5 7}$ prolateral view $\mathbf{5 8}$ retrolateral view. Figures 51-56 CASENT9040013 from M2 Cave 57, 58 CASENT9040066 from No Name Cave.


Figures 59-64. Internal anatomy of Trogloraptor marchingtoni, female (CASENT9040051) from No Name Cave. 59 vulva, dorsal view $\mathbf{6 0}$ female posterior respiratory system, tracheae and apodemes, with arrows to median apodemes 61-64 Scanning electron micrographs of the internal anatomy $\mathbf{6 1}$ vulva, dorsal view, AT, atrium, RC, receptaculum $\mathbf{6 2}$ apex of right receptaculum $\mathbf{6 3}$ posterior respiratory system, dorsal view, with white arrow to median apodeme and black arrows to lateral tracheal branches; and 64 apex of apodeme (white arrow), note frayed end typical of muscle attachment. Booklungs removed from preparation in 59, 61 and $\mathbf{6 2}$.
margin of the carapace and undulate dusky markings along the lateral carapace margin. These markings suggest that there may be at least one additional Trogloraptor species currently known only from the juvenile. Records for this specimen are as follows: CALIFORNIA: Del Norte Co., NE Crescent City, Jedediah Smith Redwood State Park, Ruth Perry Hatton Grove, US199 0.2 mi E junction with Walker Rd., elev. $60 \mathrm{~m}, 10.29 \mathrm{~km}$ NNE Crescent City, old growth redwood, under woody debris, 31 March 2011, E. Garcia, C. Richart, A. Schoenhofer, D. Sitzmann, 1 juvenile, CASENT9040069 (CAS).


Figures 65-68. Spinnerets, ventral view. 65 Segestriidae female, Ariadna sp. from Tinglewood, Western Australia (CASENT 9020550) 66 Pholcidae female, Artema atlanta from Hawaii, USA (CASENT 9047601) 67 Drymusidae female, Drymusa capensis from Table Mountain, South Africa (CASENT 9043173); and 68 Trogloraptoridae male, Trogloraptor marchingtoni (CASENT 9040065). Arrows point to membranous band on ALS base.


Figures 69-74. Scanning electron micrographs of the ALS and PLS of Trogloraptor marchingtoni, female (CASENT9039440) and male (CASENT9040066) from No Name Cave and penultimate female from M2 Cave (CASENT9040012). 69 penultimate female, right ALS, numbers refer to the three ALS segments $\mathbf{7 0}$ male right ALS $\mathbf{7 I}$ female ALS piriform gland spigots (left image flipped to appear right) 72 female major ampullate gland spigots of ALS, arrows showing individual and grouped sensillae (right image flipped to appear left) $\mathbf{7 3}$ female PLS apex showing aciniform gland spigots (left image flipped to appear right); and $\mathbf{7 4}$ female right PLS apex. AC aciniform gland spigots MAP major ampullate gland spigot(s) Nu nubbin.


Figures 75-80. Scanning electron micrographs of spinnerets of Trogloraptor marchingtoni from No Name Cave, female (CASENT9039440), male (CASENT9040066). 75 female spinneret overview (image flipped) $\mathbf{7 6}$ male spinneret overview $\mathbf{7 7}$ female PMS overview (image flipped) $\mathbf{7 8}$ female left PMS (image flipped) $\mathbf{7 9}$ female PMS close up showing aciniform gland spigots; and $\mathbf{8 0}$ male left PMS apex showing single aciniform gland spigot. AC aciniform gland spigots ALS anterior lateral spinneret $\mathbf{m A P}$ minor ampullate gland spigot(s) PMS posterior median spinnerets PLS posterior lateral spinnerets.


Figures 81-86. Scanning electron micrographs of the spinnerets of Trogloraptor marchingtoni female (CASENT9039440) and male (CASENT9040066) from No Name Cave, and penultimate female from M2 Cave (CASENT9040012). $\mathbf{8 1}$ male colulus $\mathbf{8 2}$ male epiandrum $\mathbf{8 3}$ male posterior tracheal spiracle $\mathbf{8 4}$ penultimate female right ALS $\mathbf{8 5}$ female anal tubercle; and $\mathbf{8 6}$ male left PMS and PLS spinnerets, posterior, arrows to two aciniform gland spigots on PLS. AC aciniform gland spigots MAP major ampullate gland spigot(s) PMS posterior median spinnerets PLS posterior lateral spinnerets.

## Conclusions

Western North America, especially the Klamath-Siskiyou region of northern California and southern Oregon is rich in biodiversity, particularly with respect to its endemic plants and invertebrates (Myers et al. 2000). This area is particularly notable for relicts, i.e., primitive relatives of otherwise widespread taxa. The coast redwood, Sequoia sempervirens, is a notable example. The coastal tailed frog (Ascapus truei Stejneger, 1899, As-
caphidae) and mountain beaver (Aplodontia rufa (Rafinesque, 1817)), Aplodontiidae) are considered the world's most primitive living frog and rodent, respectively (Nielson et al. 2001; Adkins et al. 2001). Primitive arachnids and myriapods also occur in the Pacific Northwest. Shear and Leonard (2003) described the new millipede family Microlympiidae for a minute species from the Olympic Mountains of Washington. Several families of Travunioid harvestman are found here (Briggs 1971). The pseudoscorpion genera Pseudogarypus (Pseudogarypidae), Oreolpium (Garypinidae) and Pseudotyrannochthonius (Pseudotyrannochthoniidae) all have relict distributions in the Pacific Northwest. These are also among the most ancient of pseudoscorpion lineages (Harvey 1998; Harvey and Štáhlavský 2009). Atypoid mygalomorphs (Coyle 2005a, 2005b; Ramirez and Chi 2004), Hypochilidae (Hedin 2001; Paquin and Hedin 2005), the 8-eyed caponiid Calponia (Ubick, 2005) and the cribellate leptonetid genus Archoleptoneta (Ledford and Griswold 2010) comprise relatively ancient spider lineages characterized by the retention of ancestral character states. If Trogloraptoridae are the most primitive living members of the Dysderoidea we have another case of a notable relict from Western North America. If such a large and bizarre spider could have gone undetected for so long, who knows what else may lurk undiscovered in this remarkable part of the world.

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