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Laimosemion paryagi (Cyprinodontiformes: Aplocheiloidei: Rivulidae), a new species from the upper Mazaruni river drainage of Western Guyana

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Abstract

Laimosemion paryagi, new species, is described from the upper Mazaruni river system, a tributary of the Essequibo River. It is a member of the *Laimosemion breviceps* group former known as the *Rivulus breviceps* group, and shares a robust body and deep caudal peduncle with *Laimosemion breviceps* (Eigenmann, 1909) and *Laimosemion lyricauda* (Thomerson et al., 1991) and to a lesser degree with *L. gransabanae* (Lasso et al., 1992) and *Laimosemion torrenticola* (Vermeulen & Isbrücker, 2000). These species are all endemic to the Guiana Highlands in western Guyana and the neighbouring Gran Sabana in Eastern Venezuela. It is distinguished from other species in the *L. breviceps* group by morphology and its remarkable male color pattern of red blotches on a turquoise ground color on the flanks and in the unpaired fins and having a rounded caudal fin as opposed to one having extension or being spade shaped. Analysis of mitochondrial DNA sequence reveals that it is genetically distinct from all other members of this group and that inhabitants of the Guyana highlands diverged from each other early in the history of the genus, commensurate with the geological age of the Guiana Shield.

Zusammenfassung

Laimosemion paryagi nov. spec. wird vom Flusssystem des oberen Mazaruni beschrieben, einem Zuflusssystem des Essequibo. Diese neue Art gehört zur *Laimosemion-breviceps*-Gruppe, die früher *Rivulus-breviceps*-Gruppe genannt wurde, und hat einen robusten Körper und einen tiefen Schwanzstiel mit *Laimosemion breviceps* (Eigenmann, 1909) und *Laimosemion lyricauda* (Thomerson et al., 1991) gemeinsam, in geringerem Grade auch mit *L. gransabanae* (Lasso et al., 1992) and *Laimosemion torrenticola* (Vermeulen & Isbrücker, 2000). All diese Arten sind endemisch im Guyana-Hochland in West-Guyana sowie im benachbarten Gran Sabana in Ost-Venezuela. Die neue Art unterscheidet sich von den anderen Arten der *L.-breviceps*-Gruppe durch die Körpergestalt und das auffällige Farbmuster der Männchen: rote Flecken auf türkisfarbenem Grund an den Flanken und den unpaaren Flossen sowie eine abgerundete Schwanzflosse, während die anderen Arten mit Fortsatz oder spatenförmigen Schwanzflossen ausgestattet sind. Eine Analyse der mitochondrialen DNA-Sequenz zeigt, dass die neue Art sich genetisch von allen anderen Angehörigen der Gruppe unterscheidet und dass die Bewohner des Guyana-Hochlandes als Divergenz zu allen anderen Arten in der Frühzeit der Gattungsgeschichte entstanden sind, gemessen am geologischen Alter des Guyana-Schildes.

Résumé

Laimosemion paryagi, nouvelle espèce, est décrit comme originaire du système du haut Mazaruni, un tributaire de l'Essequibo River. C'est un membre du groupe *Laimosemion breviceps*, jadis groupe *Rivulus breviceps*, et partage un corps robuste et un large pédoncule caudal avec *Laimosemion breviceps* (Eigenmann, 1909) et *Laimosemion lyricauda* (Thomerson et al., 1991) et, à un moindre degré, avec *L. gransabanae* (Lasso et al., 1992) et *Laimosemion torrenticola* (Vermeulen & Isbrücker, 2000). Ces espèces sont toutes endémiques des hauteurs de l'ouest de la Guyana et du Gran Sabana voisin, à l'est du Venezuela. Il se distingue des autres espèces du groupe *L. breviceps* par la morphologie et par le remarquable patron de coloration du mâle fait de taches rouges sur fond turquoise sur les flancs et les nageoires impaires et par une caudale arrondie au contraire de celle de forme étendue ou en pelle. L'analyse de séquence mitochondriale d'ADN révèle que l'espèce se distingue génétiquement de tous les autres membres de ce groupe et que les espèces des hauteurs de Guyana diffèrent l'une de l'autre tôt dans l'histoire du genre, proportionnellement à l'âge géologique du Bouclier guyanais.

Sommario

Laimosemion paryagi, nuova specie, è descritto dal sistema superiore del fiume Mazaruni, un affluente del fiume Essequibo. Si tratta di un membro del gruppo *Laimosemion breviceps* un tempo noto come gruppo *Rivulus breviceps*, e condivide con *L. breviceps* (Eigenmann, 1909), *L. lyricauda* (Thomerson et al., 1991) e, in misura minore, con *L. gransabanae* (Lasso et al, 1992) e *L. torrenticola* (Ver-

meulen & Isbrücker, 2000), un corpo robusto e un altrettanto robusto peduncolo caudale. Queste specie sono tutte endemiche delle Guiana Highlands nella parte occidentale della Guyana e della vicina Gran Sabana nel Venezuela orientale. Si distingue dalle altre specie del gruppo *L. breviceps* per la notevole colorazione del maschio, fatta di chiazze rosse su un fondo di colore turchese sui fianchi e sulle pinne impari, e per la morfologia tra cui una pinna caudale arrotondata rispetto ad una avente estensione o a forma di vanga. L'analisi della sequenza di DNA mitocondriale rivela che è geneticamente distinta da tutti gli altri membri di questo gruppo e che le specie che abitano gli altopiani della Guyana si allontanarono uno dall'altro piuttosto presto nella storia del genere, commisurato con l'età geologica dello scudo della Guiana.

INTRODUCTION

The recently erected genus *Laimosemion* (Cyprinodontiformes: Aplocheiloidei: Rivulidae) currently includes twenty four species (Costa, 2011). It represents a molecularly defined clade (Murphy et al., 1999; Hrbek and Larson, 1999; Hrbek et al., 2004; Suijker and Collier, 2006) within a large, diverse assemblage of species (formerly assigned to the genus *Rivulus)* that are distributed throughout Northwestern South America; central and eastern Brazilian Amazon; river basins of Guianas; upper Orinoco River basin in Venezuela and Colombia; Rio Negro basin in Brazil; and lower Peruvian Amazon. They are found in coastal lowland areas as well as mountainous terrain occasionally up to altitudes of 1300 meters above sea level. Within these areas they are found in small streams, creeks, swampy or wet places adjacent to creeks and rain pools.

Past authors have attempted to arrange related forms of this larger group into a number of complexes or species groups or subgenera (Hoedeman, 1958 and 1961; Huber, 1992 and 1999; Costa, 2006). Hoedeman (1958) coined the term "breviceps complex" for "small forms, coarsely scaled" which included *breviceps*, *frenatus*, *agilae* and *geayi*. Huber (1999) grouped these species into the subgenus *Laimosemion* based on small size, distinctive male patterns, a red opercular shield and that two had been reported to have bifid epipleural ribs, a unique character among the Rivulidae. Costa (2006) refined the morphological and osteological definition of this subgenus and erected a new subgenus *Owiyeye* for a distinctive set of related species from west of the Guiana Shield. Subsequently, Costa (2011) elevated the subgenus *Laimosemion* to genus and redefined it to also include those species he formerly included in *Owiyeye* primarily on the basis of previously published molecular phylogenies (Hrbek and Larson, 1999; Murphy et al. 1999: Hrbek et al., 2004; and Suijker and Collier, 2006).

The first and second authors have visited Guyana several times since 1989. These series of expeditions were the first serious attempts to relocate all species of the former genus *Rivulus* described by Eigenmann (1909 and 1912) based upon material collected during his expedition of 1908. During these and other expeditions to the neighboring areas of Venezuela, five additional species have been described that can be included in this group of species (Thomerson at al., 1991; Lasso et al., 1992; Vermeulen and Isbrücker, 2000; Lasso-Alcala et al., 2006: Suijker and Collier, 2006). The new species presented in this paper was first collected during a survey of the Guyana plateau by W. H. Suijker and Y. Suijker, accompanied by S. C. Paryag on April 4, 1995.

MATERIAL AND METHODS

Specimens analysed: Wild-caught specimens were used as holotype and paratype while first generation aquarium bred descendents of wild-caught fish were used for other paratypes, preparations of karyotypes and DNA extractions. Specimens of *Laimosemion gransabanae* from San Rafael de Kamoirán, Venezuela, *Laimosemion breviceps* from Kaieteur Falls, Guyana and *Laimosemion torrenticola* from Kamarang, Guyana, were also used for DNA extractions.

Molecular analyses: DNA was extracted from ethanol preserved specimens using a DNAeasy kit (Qiagen). Amplification of portions of the mitochondrial genes for 12S rRNA, 16S rRNA, and cytochrome B was done as previously described (Murphy and Collier, 1996, and 1997. GenBank accession numbers for the taxa used are: *L. geayi* (AF002433, AF002537, AF002483), *L. strigatus* (AF002434, AF002538, AF002484), *L. agilae* (AF002432, AF002536, AF002482), *L. frenatus* (AF002435, AF002539, AF002485), *L. xiphidius* (AF002436, AF002540, AF002486), *L. lyricauda* (AF002439, AF002543, AF2489), *L. rectocaudatus*(AF002440, AF002544, AF002490), *L.* sp. Tobogan (AF002437, AF002541, AF002487), *L.* sp. DCT89-132 (AF002438, AF002542, AF002488), *L. mahdianensis* (DQ501248, DQ501249, DQ501250), *R. cylindraceus* (U41799, AF002533, U41781) and *R. roloffi*

(U41798, AF002434, U41780). The GenBank accession numbers for *L. breviceps* and *L. paryagi* are JX885658 through JX885663.

Sequences were aligned by Clustal W and analyzed using MEGA 4.1 (Tamura et al., 2007).

Karyotype: Metaphase preparations of chromosomes were prepared from gill epithelium of a single wild-caught male as described by Kligerman and Bloom (1977).

Morphometrics: Measurements and counts follow Huber (1992). All visible and minute rays from the anal and dorsal fins were counted. Measurements are taken with a Mitutoyo Dial Calliper to the nearest tenth of a millimetre and are presented in percentages of the standard length.

Clearing and staining of a specimen was performed as described by Taylor and Van Dyke (1985).

Laimosemion paryagi, n. sp.

Figs 1-2, Table I

Holotype: ZMA (Zoölogisch Museum Amsterdam) 124.896, male, 39,2 mm SL: Guyana, Upper Mazaruni District, upper Mazaruni river about 7

Fig. 1. *Laimosemion paryagi* n. sp. (not preserved). Male from the terra typica. Photo by F. Vermeulen.

Fig. 2. *Laimosemion paryagi* n. sp. (not preserved). Female from the terra typica. Photo by F. Vermeulen.

miles downstream from Kamarang, a little creek about 15 minutes walk on a bush trail in eastern direction starting at the right bank near a rapid locally called "Sand Landing" (Figures 3 and 4). Coordinates: 05°43'50"N – 60°30'55" W, altitude approx. 495 meters above sea level. April 4, 1995. Paratypes: ZMA 124.897, one female, 36,9 mm SL: Collected same locality and date as holotype and 6 males F1 generation, original from same location.

Diagnosis: *Laimosemion paryagi* is a member of the *L. breviceps* species group as indicated by its relative small size, short snout length, bright coloration and relatively low number of scales (LL+C). It is distinguished from lowland close relatives *(L. agilae, L. xiphidius, L. cladophorus, L. geayi* and *L. mahdiaensis)* by having a deeper caudal peduncle (14.1-17.3 % of SL, vs. 11.5-13.6 % of SL). It is distinguished from the remaining species in the group by having a rounded caudal fin (versus a spade-shaped caudal fin in *L. breviceps, L. gransabanae* and *L. torrenticola*, and a lyre-shaped caudal fin in *L. lyricauda)*. Its remarkable coloration of red blotches on a turquoise background on the flanks and in all unpaired fins in males (versus absence of turquoise color and red blotches) is unique within the group.

Fig. 3. The habitat with the tea colored water lighting up in the sun. *L. paryagi* was not seen in the creek itself but in swampy places beside the creek. Photo by S. Sladkowski.

Description: Morphometric data are presented in Table I. Largest specimen 39.2 mm SL, male. The dorsal outline of the body has a weak convex profile, straight from anterior of the dorsal fin along the caudal peduncle. Ventral profile convex, to the caudal peduncle more straight. Body cylindrical. Greatest body depth at level of pelvic fin insertion. Dorsal fin short triangular shaped, no filaments in either males or females, somewhat larger in males. Dorsal fin origin directly above 6th or 7th anal fin-ray. Anal fin rectangular in males, more rounded or slightly rectangular in females, no fin rays extended as filaments. Caudal fin rounded in both sexes. Pelvic fins rounded, in males reaching the second ray of anal fin, in females smaller not reaching anal fin. Pectoral fins in males somewhat larger than in females but both not reaching base of pelvic fin. Dorsal fin-rays 9; anal fin-rays 11-12; pelvic fin-rays 6.

Scales large and cycloid, head scaled except the ventral part of cheeks. Longitudinal series of scales 31-32 upon the end of lateral plate, some scales (3-5) on the caudal fin base, no scales on base of other fins. Frontal scales are circularly arranged around the central A-scale. The squamation pattern is of the E type in most samples and of the E-D type in 2 of the 8 samples. Scales along the mid- lateral line have single minute sensory organs.

Epipleural ribs are not bifid (Fig. 8).

Coloration in life: Male: Dorsum brown to dark brown, laterally the body is light brown with aubergine red spots arranged to form weak and broken lines, more intense on the anterior portion. These clear, visible red spots are shown only on the upper half of the sides and are only weakly present on the lower half. The lower half of the sides is iridescent turquoise from the lower edge of the eye, over the gill-cover, to the end of the caudal peduncle. Venter and cheeks are whitish to grey. Iris of the eye is golden. No opercular or humeral markings are present. The side of the head is orange to yellow. The dorsal fin is turquoise with large aubergine- red blotches that tend to form

Table I. Morphometric data of the holotype and paratypes of *Laimosemion paryagi*. Standard length is given in mm, other morphometric data are presented as percentages of standard length. The abbreviation "dev" = deviation.

	Holotype	Paratypes							8 samples			
	male	female	male	male	male	male	male	male	lowest	highest	average	dev
Standard length in mm	39.2	36.9	28.1	26.0	23.0	26.7	35.2	36.9	23.0	39.2	31.5	5.8
In % of SL												
Total length	124.0	110.8	119.8	120.9	124.9	119.6	114.7	120.7	110.8	124.9	119.4	4.4
Pre-dorsal length	69.4	69.4	71.7	69.0	73.6	71.3	70.2	69.4	69.0	73.6	70.5	1.5
Pre-anal length	62.0	59.2	58.4	62.6	59.8	60.0	59.7	61.4	58.4	62.6	60.4	1.4
Body depth at anal level	23.0	20.8	22.1	24.7	22.3	23.9	19.8	23.9	19.8	24.7	22.6	1.5
Head length	25.7	21.9	26.2	26.5	27.9	25.8	20.9	24.9	20.9	27.9	25.0	2.2
Snout length	4.1	3.0	5.7	6.5	4.5	4.2	5.2	5.1	3.0	6.5	4.8	1.0
Interorbital space	11.5	12.0	12.7	10.0	12.5	11.7	11.9	13.4	10.0	13.4	12.0	0.9
Eye diameter	7.9	7.1	9.4	7.4	10.5	8.5	7.9	8.5	7.1	10.5	8.4	1.0
Depth of caudal peduncle	15.3	14.7	16.0	17.4	15.3	14.9	14.1	16.5	14.1	17.4	15.5	1.0
Snout to dorsal	69.4	69.4	71.7	69.0	73.6	71.3	70.2	69.4	69.0	73.6	70.5	1.5
Snout to anal	62.0	59.2	58.4	62.6	59.8	60.0	59.7	61.4	58.4	62.6	60.4	1.4
2. Meristic data												
Number of dorsal fin rays	9.0	9.0	9.0	9.0	9.0	9.0	9.0	10.0	9.0	10.0	9.1	0.3
Number of anal fin rays	13.0	12.0	12.0	12.0	13.0	13.0	13.0	13.0	12.0	13.0	12.6	0.5
Dorsal insertion to anal insertion	7.0	6.5	6.5	6.0	6.5	6.5	7.0	7.0	6.0	7.0	6.6	0.3
Longest dorsal fin ray	6.0	4.4	4.2	3.7	3.5	3.7	4.2	6.3	3.5	6.3	4.5	1.0
Longest anal fin ray	7.1	3.1	4.6	4.0	3.6	4.0	4.6	6.0	3.1	7.1	4.6	1.2
3. Scalation												
Number scales LL + C	32.0	32.0	32.0	33.0	32.0	34.0	32.0	32.0	32.0	34.0	32.4	0.7
Number scales to D insertion	22.0	22.0	22.0	22.0	23.0	22.0	22.0	22.0	22.0	23.0	22.1	0.3
Head scalation type	E	E	E	E	E	damaged	D-E	E-D				

lines along the rays, while the distal portion of the dorsal fin fades to more red than turquoise to form a marginal aubergine- red zone. Anal fin is bluish to turquoise near the base with some weak red blotches. The distal two thirds of the anal fin becomes more red than turquoise to form a marginal aubergine- red zone. Caudal fin basis is turquoise, with the center more iridescent and the edges of which fade to form an aubergine-red margin around the outer edge. The central part of the caudal has rows of numerous red dots that form 3-4 vertical bars. The pelvic fin is almost completely aubergine-red with a weak bluish at the base. Pectoral fin is hyaline orange.

Female: Dorsum light brown, pale brown on the sides and yellow brown to the vent. The anterior part of the flanks show hyaline red markings. Dorsal fin is hyaline orange with weak darker dots forming horizontal lines. Anal fin is hyaline orange with more orange toward the distal end. Caudal fin is hyaline orange without any markings or marginal band. Pelvic fins hyaline. Pectoral fins hyaline orange. Eye with golden iris.

No "Rivulus spot" or supracaudal ocellus is present in either sex or at any stage of life. There is a dark (black) stripe that appears below the midline from behind the pectoral fin to nearly the base of the caudal. This is seen in both sexes when they are stressed.

Behaviour: In general, species that were formerly placed in the genus *Rivulus* (now divided into *Anablepsoides*, *Atlantirivulus*, *Cynodonichthys*, *Kryptolebias*, *Laimosemion*, *Melanorivulus* and *Rivulus* by the classification as proposed by Costa, 2011), are non-schooling, solitary fish that are found in very small streams and swamps. In our collecting experience, they are found in the dense vegetation of swamps, under rocks or between logs and leaf litter in cascading mountain creeks. Often they can be found in the wet leaf litter adjacent to small streams. As a result they are often missed in general

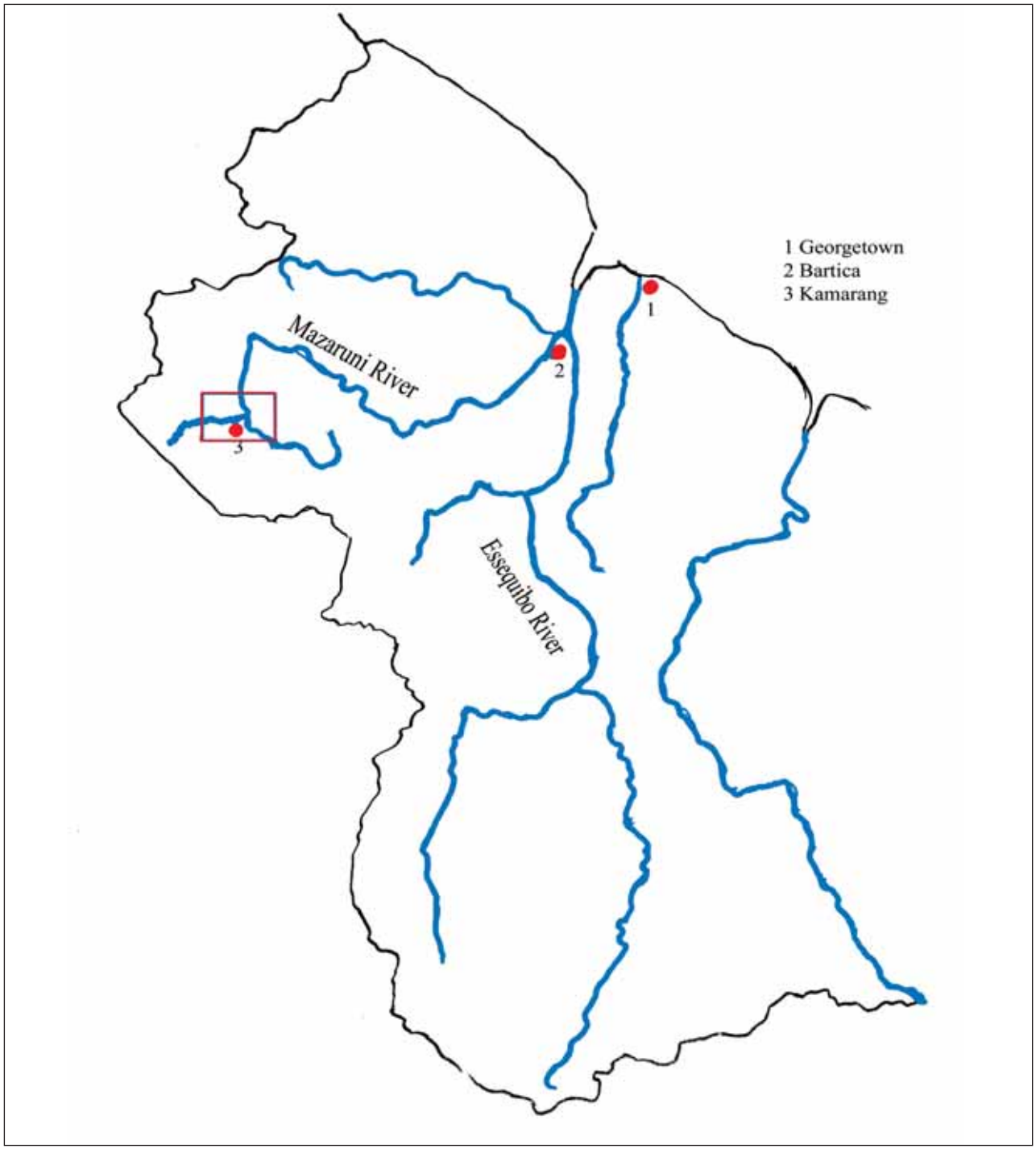

Fig. 4. Main river systems of Guyana.

ichthyological surveys of larger fishes or larger bodies of water. They are most often discovered by coincidence during expeditions with other objectives. Collectors of *Laimosemion* almost never see the species that they are looking for until they see the fish in their nets after "blind" collecting.

Members of the *L. breviceps* species group display behaviour that is an exception to the general pattern described above for the larger group of related genera. Although they do not school, they are clearly seen not hiding and in open water. This behaviour, witnessed in the field by authors in many occasions, is also shared by *Anablepsoides waimacui* and *Anablepsoides amphoreus*. All members of the *Laimosemion breviceps* group, as well as the two species mentioned above, do not share their habitat with any other fish species. The lack of fish predators may be responsible for this remarkable behaviour.

Molecular phylogeny: Sequences of portions of the mitochondrial genes for 12S and 16S RNA and cytochrome b for *L. paryagi* were added to those previously determined (Murphy et al., 1999; Suijker and Collier, 2006). The resulting phylogeny places the new species as a member of this group with the closest relationship being to *L. breviceps*. Partial DNA sequences for *L. gransabanae* and *L. torrenticola* place them as close relatives of *L. breviceps* as well.

Karyotype: Metaphase spreads (Figure 7) reveal 2n=26. The haploid complement consists of one large metacentric and one medium size metacentric element and a graded series of smaller metacentric and submetacentric elements. This is the lowest number recorded to date for a species of the genus *Laimosemion*. Low diploid numbers also characterize other members of the group shown in Figure 6 (Suijker and Collier, 2006).

Distribution: Known only from the type locality, a small black- water creek, about 15 minutes walk on a hunting trail starting at the right bank of the upper Mazaruni river about 10 km downstream from Kamerang near rapids locally called "Sand Landing".

Etymology: The name *paryagi* is chosen to honour Mr. Subhas Chand Paryag, from Georgetown, Guyana, co-collector of the new species and local helper during most of the expeditions in Guyana made by first and second author.

Discussion: *Laimosemion paryagi* is a member of the *L. breviceps* species group as indicated by its relative small size, short snout length and bright

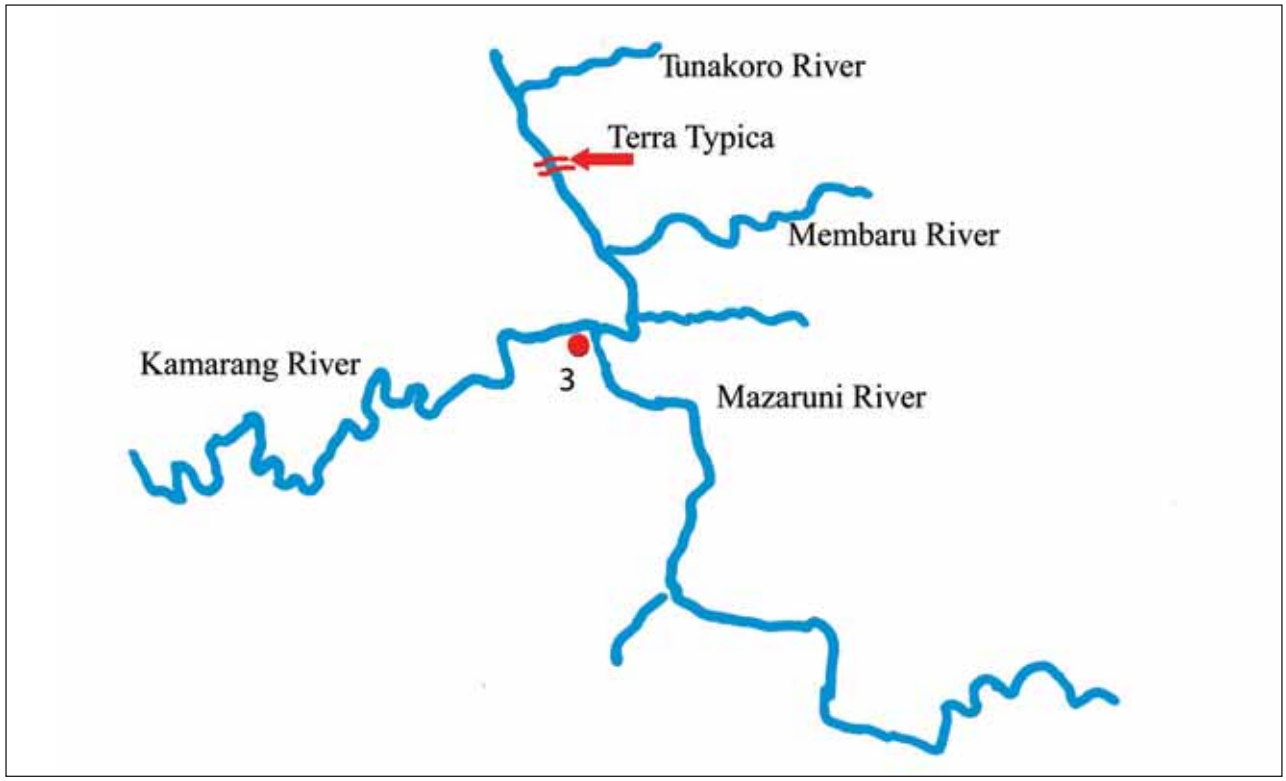

Fig. 5. Upper Mazaruni River at Kamarang.

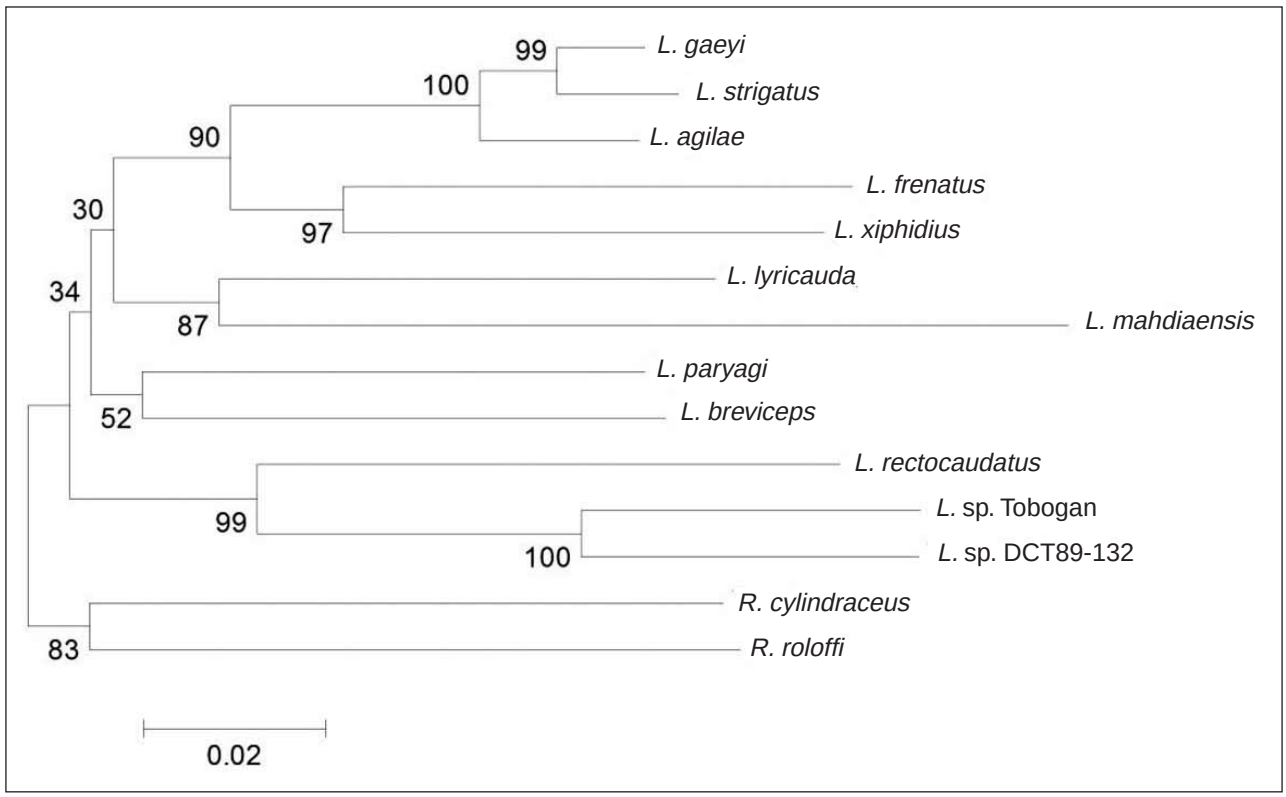

Fig. 6. Molecular phylogeny for *L. paryagi* and selected members of the genus *Laimosemion.* Neighbor-joining tree based upon 1048 base pairs of portions of the cytochrome *b*, 12S, and 16S mitochondrial genes. The values below the nodes are bootstrap values. *Rivulus cylindraceus* and *Rivulus roloffi* were used as outgroups to root the tree. Previous subgeneric groupings (Costa, 2006) and current generic assignment (Costa, 2011) are indicated by brackets.

coloration. It is distinguished from other members of the group principally by the shape of caudal fin and a unique color pattern. This placement is also supported by the molecular and cytogenetic data. The dark line displayed below the midline when stressed is a character that is shared with *L. breviceps*, *L. agilae* and *L. lyricauda*. This unique, shared color pattern may be another character that unites the group.

This group has also been assigned to the subgenus *Laimosemion* (Huber, 1999; Costa, 2006). However, the primary osteological diagnostic characteristic of this subgenus was given as "bifid epipleural ribs" (Costa, 2006). The subgenus *Owiyeye* was erected (Costa, 2006) to include species from west of the Guiana Shield. In Figure 6 this subgenus is represented by *L. rectocaudatus*, *L.* sp. Tobogán and *L.* sp. DCT 89-132 (Maroa). This subgenus is characterized by, among other characters, "frontal scales transversely arranged" and "squamation S-patterned". None of these characters are found in *L. paryagi*. Thus, *L. paryagi* cannot be assigned to either of these subgenera as defined by Costa (2006).

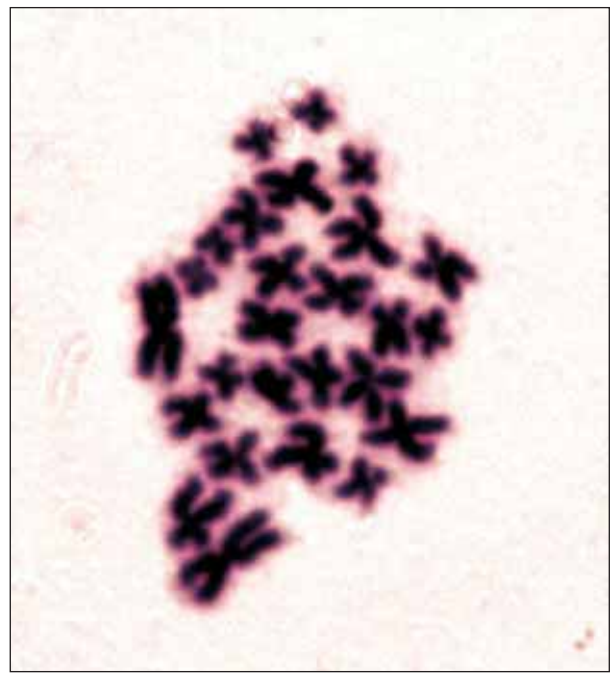

Fig. 7. Metaphase chromosomes from gill epithelia of *Laimosemion paryagi.*

Costa (2011) conceded that the morphological characters had not been fully examined in all species of the subgenus *Laimosemion* and recognized the monophyly of the set of species included in the subgenera *Laimosemion* and *Owiyeye* revealed by previous published molecular phylogenies. Accordingly *Laimosemion* was raised to generic rank to include all species of the two former subgenera.

However, the species of *Owiyeye* differ from the other species in the new genus in their size, behaviour and, most importantly, geographical distribution. Unfortunately, most species formerly assigned to *Owiyeye* have not been made available for molecular analysis. Until there can be a comprehensive analysis of both morphological and molecular characters, the taxonomic status of these species will remain ambiguous. For these reasons, we prefer to informally use the term breviceps group to refer to those small species found along the eastern edges of the Guiana Shield and adjacent lowlands where they are found in creeks and wetland areas with stony or sandy bottoms.

The Guiana Shield is an ancient geological formation that forms one of the three cratons of the South American plate. The Guiana highlands are the higher elevations of this formation found in west central Guyana. Some of the most spectacular waterfalls in world flow off these highlands to the surrounding lowlands. These highlands were presumably never inundated by epicontinental seas. Hence species of freshwater fishes endemic to this region likely diversified over a longer period of time than species from the surrounding lowlands. The molecular phylogeny is consistent with this notion in that all members from the interior and highland areas of the Guiana Shield are deeply diverged from one another. Even though these species are each other's closest relatives, they represent a diversity of morphology and coloration unparalleled in other parts of the former genus *Rivulus*. Whether this is simply a function of the age of the Guiana Shield or other factors remain to be seen.

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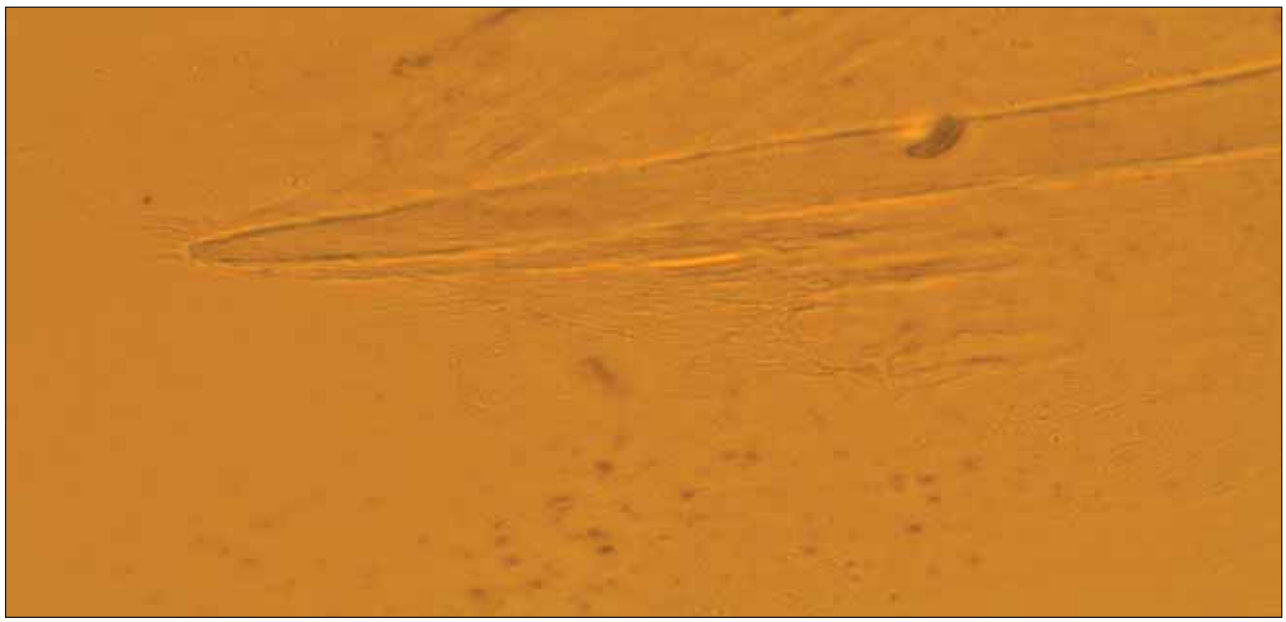

Fig. 8. Fifth epipleural rib dissected from the right side of a cleared and stained specimen of *L. paryagi.*

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