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Ohio USA stoneflies (Insecta, Plecoptera): species richness estimation, distribution of functional niche traits, drainage affiliations, and relationships to other states

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Abstract

Ohio is an eastern USA state that historically was >70% covered in upland and mixed coniferous forest; about 60% of it glaciated by the Wisconsinan glacial episode. Its stonefly fauna has been studied in piecemeal fashion until now. The assemblage of Ohio stoneflies was assessed from over 4,000 records accumulated from 18 institutions, new collections, and trusted literature sources. Species richness totaled 102 with estimators Chao2 and ICE Mean predicting 105.6 and 106.4, respectively. Singletons and doubletons totaled 18 species. All North American families were represented with Perlidae accounted for the highest number of species at 34. The family Peltoperlidae contributed a single species. Most species had univoltine–fast life cycles with the vast majority emerging in summer, although there was a significant component of winter stoneflies. Nine United States Geological Survey hierarchical drainage units level 6 (HUC6) were used to stratify specimen data. Species richness was significantly related to the number of unique HUC6 locations, but there was no relationship with HUC6 drainage area. A nonparametric multidimensional scaling analysis found that larger HUC6s in the western part of the state had similar assemblages with lower species richness that were found to align with more savanna and wetland habitat. Other drainages having richer assemblages were aligned with upland deciduous and mixed coniferous forests of the east and south where slopes were higher. The Ohio assemblage was most similar to the well–studied fauna of Indiana (88 spp.) and Kentucky (108 spp.), two neighboring states. Many rare species and several high quality stream reaches should be considered for greater protection.

Keywords
Plecoptera, Stonefly, Biodiversity, Checklist, Ohio, North America

Introduction

Regional biodiversity studies are of great importance for setting conservation priorities, in determining conservation status of species, and in examining factors that govern diversity (de Silva and Medellín 2001). The resulting species lists help other professionals to know what species live in the region. This is especially important for ecologists who use species and assemblage characteristics as water quality indicators. Conservation agencies can use these checklists and ecological relationships to help prioritize conservation initiatives, including the rehabilitation of habitat, purchase of land, planning for reintroductions, and establishment of imperilment risk for various taxa.

Ohio is an eastern state of the USA with a total area of 105,910 km^2. It is bound on the south and east by the Ohio River and drained by 10 United States Geological Survey six digit scale Hydrologic Drainage Units (USDA 2009, HUC6s) (Fig. 1). The river and its tributaries have served as conduits for westward and northward migration from Kentucky, Pennsylvania, and West Virginia after the most recent glacial event

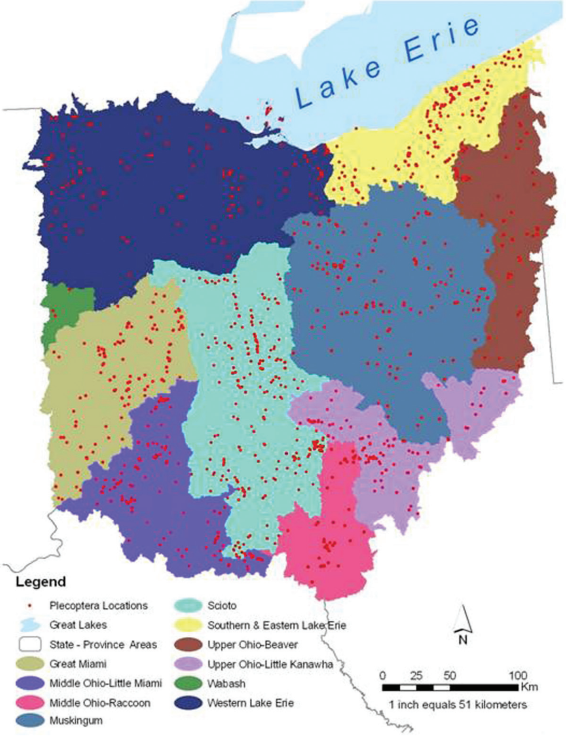

Figure 1. HUC6 drainages and point locations for Ohio Plecoptera collections.

cleared much of the fauna from the state. The Wisconsinan glaciation retreated 18,000 years bp, leaving a highly modified landscape. Areas in the northwest two–thirds of the state were heavily glaciated, leaving till (west central), lake (NW), and drift (NE) plain landscapes. Southeast of this line is found the unglaciated Allegheny plateau, an area of deeply incised hills and valleys filled with glacial outwash. Pre-European settlement land cover in Ohio was dominated by upland forest (71%), wooded wetland (13.8%), and mixed deciduous and coniferous forest (Fig. 2), the overall similarity to the Appalachian forests diminishing rapidly westward. Ohio's human population in 2010 was 11,536,504 (USCB 2011).

Plecoptera (stoneflies) are known the world over as being environmentally sensitive (Fochetti and Tierno de Figueroa 2008). Because of this, they have been used extensively as indicators of water quality (Lenat 1993, Stewart and Stark 2002). Imperilment of significant numbers of species has been demonstrated in Europe (Zwick 1992), in the United States (Master et al. 2000), and for the USA state of Illinois (DeWalt et al. 2005).

Ohio's stonefly fauna has been studied in a piecemeal fashion. Walker (1947) provided the first list of species, but covered only southeastern Ohio. Very few of his specimens survive today and most records were published only at the county level. Shortly thereafter, Gaufin (1956) updated the list from material collected from the southwestern quarter of the state. Many of his specimens were donated to the Monte L. Bean Museum at Brigham Young University. Several studies of a narrower scope

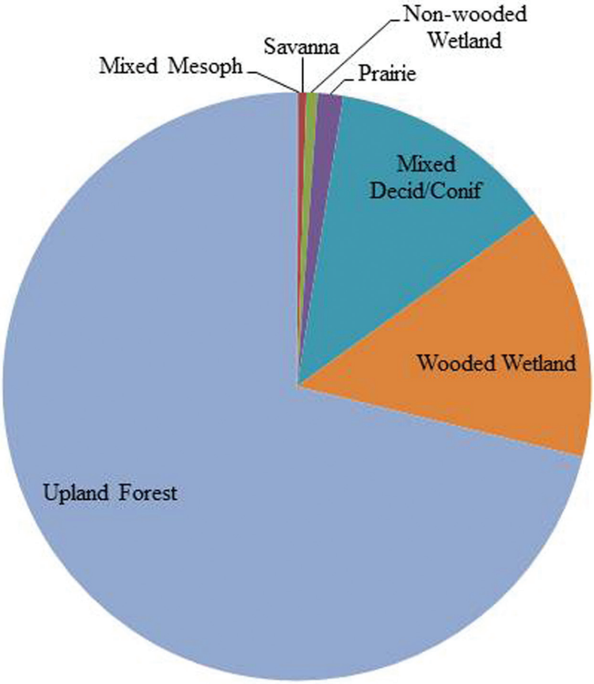

Figure 2. Pre-European settlement vegetation percentage cover for Ohio (from Ohio Department of Natural Resources 2003).

were published for individual streams in northeastern Ohio in the 1970s and 1980s. Tkac and Foote (1978) published on the fauna of Stebbins Gulch in Geauga County, while Robertson (1979, 1984) published on Penitentiary Glen of Lake County. Fishbeck (1987) reported on stoneflies inhabiting Gray's Run of Mahoning County and Beckett (1987) studied the nymphs of stoneflies inhabiting the polluted Ohio River at Cincinnati, Ohio. Additional records have appeared in several recent works including Stark (2004), Surdick (2004), Kondratieff (2004), and Kondratieff and Kirchner (2009). The last large undertaking was conducted by Tkac (1979) as part of his unpublished doctoral dissertation on the northeastern Ohio fauna. Relatively few Tkac specimens have been located; some may be in the United States National Museum.

The coauthors have embarked on a study of the stonefly fauna of the Midwest, including distribution modeling of up to 160 species known from Illinois, Indiana, Michigan, Ohio, Ontario, and Wisconsin in order to reconstruct pre-European settlement range. Given that there has been no comprehensive assessment of the stonefly assemblage in Ohio we have elected to prepare one. This analysis is based on the accumulated specimen records from our own efforts, the efforts of colleagues over several decades, the examination of nearly 30,000 specimens borrowed from regional museums, and reliable literature records. We ask several questions of these data:

- How many stonefly species inhabit Ohio?
- How completely has the fauna been sampled?
- How are functional niche traits distributed across the assemblage?
- Does drainage affiliation affect assemblage composition and species richness?

Methods

Specimen Records

Specimens are the only resource where identifications may be verified, so the study was based on an abundance of specimens examined from 18 regional museums (Table 1). Pinned specimens were relaxed in a humid chamber and the terminalia cleared in 10% KOH. Cleared terminalia were acidified in dilute acetic acid, rinsed in water, and stored in glycerin under the specimens. Eggs were removed before clearing and rehydrated to view ultrastructural characters. Eggs were also stored in glycerin below the specimen. Adults in alcohol were dealt with similarly, the eggs and abdomens stored in shell vials inside the larger vial. Each specimen or series in a vial was associated with a database record using a paper catalog number. Label data and carefully scrutinized literature records were added to a local database. The raw data set is available as "Appendix 1 Raw Specimen data" in Excel Spreadsheet format. A glossary to Appendix 1 has also been provided as supplementary data.

Table 1. Specimen origin, institutional coden, number of specimen records, and number of specimens examined.

Institution	Coden	#Records	Specimens
Brigham Young University	BYU	1167	18811
B. P. Stark Collection	BPSC	6	81
Canadian National Collection	CNC	46	252
Cincinnati Museum of Natural History	CMNH	2	2
Cleveland Museum Natural History	CLEV	66	171
Field Museum Natural History, Chicago	FMNH	13	40
Illinois Natural History Survey	INHS	639	2839
Michigan State University	MSUC	11	63
Ohio Biological Survey	OBS	573	2690
Ohio Environmental Protection Agency	OEPA	83	142
Ohio Historical Society	OHSC	17	17
Ohio State University	OSU	468	668
Purdue University	PURC	7	18
R. Fred Kirchner Collection	RFKC	164	857
Royal Ontario Museum	ROME	3	15
Southern Illinois University Carbondale	SIUC	1	5
University of Michigan	UMMZ	3	3
Western Kentucky University	WKU	170	873
Literature	Author Year	641	4940
Total		4,080	32,487

New specimens were collected using sweep nets, beating sheets, hand picking, and dipnetting throughout the state. Many nymphs were reared in Styrofoam cups or in a Frigid Units Living Stream at the University of Illinois. Illinois Natural History Survey (INHS) specimens collected after 2007 were preserved in 95% EtOH and stored in a –20C freezer for future molecular studies.

Locations for all specimens were georeferenced to the finest scale permitted by the label data. Coordinate precision for each record was marked as a radius about the location: from GPS = code 1(10 m); post–processed with small town or road crossing and stream name = code 2 (1,000 m); town name only or large town with stream name = code 3(10,000 m), county level record = code 4 (100,000 m); state level record = code 5 (1,000,000) m. Only records with codes 1–3 were used for species accumulation curves and nonparametric multidimensional scaling (NMDS) analyses.

Data Analysis

How many stonefly species inhabit Ohio and adequacy of sampling? These are complex questions that we answered in two ways. First, a list of species was tallied from all specimen data. All data were used for this purpose. Second, records with precision code 1–3 were used to estimate species richness using the program EstimateS v8.2 (Colwell 2006). Museum data are biased due to the inability to quantify the sampling effort for every researcher. To reduce this bias, we elected to use unique locations as sample units, regardless of date of collection. In this way, the number of sites with multiple visits is maximized. This is important for stoneflies due to their proclivity for a succession of species throughout the year and because the nymphs for many species are unidentifiable (Stewart and Stark 2002). Unique locations are presented as "Appendix 2 OH Unique Locations". Raw data were summarized to produce a species presence/absence by unique location data matrix, available as "Appendix 3 spp. Location Matrix". The data matrix was analyzed using 50 randomizations, strong hash encryption, and randomization without replacement. Cumulative assemblage richness (Sobs–Mao Tau), the number of singletons and doubletons, and two estimators of species richness (Chao2 and ICE Mean) were plotted versus unique locations. Comparison of species richness and assemblage composition with Illinois, Indiana, Kentucky, Michigan, Ontario, Pennsylvania, Wisconsin, and West Virginia was conducted by compiling species lists from these states using only published records compiled in DeWalt et al. (2011) and Nelson (2008). A species by state/province matrix was constructed and a Sørensen Index of Similarity of each pairwise comparison calculated using EstimateS (Colwell 2006).

How are species traits distributed across the assemblage? Poff et al. (2006) provided a summary of functional niche traits for several life history, mobility, morphological, and ecological traits for aquatic insects. These were expressed at family and generic taxonomic levels and are useful in characterizing large scale ecological and evolutionary conditions under which a species or an assemblage lives. Stonefly functional niche traits are perhaps the best known of all aquatic insects in North America (see vast summaries of Stewart and Stark 2002 and references therein). We compiled a subset of Poff et al. (2006) functional niche traits and, to the best of our ability, recorded trait states for all species known to occur in Ohio (Table 2). These trait states were drawn from numerous literature sources (Harper 1973a, b, Harper and Hynes 1972, MacKay 1969, Stewart and Stark 2002) and from 30 years of professional experience working in the Midwest region. Of course, life histories and detailed feeding studies were not available for all species, so trait states for many species were surmised from closely related taxa. The number of species thought to have each trait state was tallied for the entire Ohio assemblage.

Does drainage affiliation affect assemblage composition and species richness? HUC6 units were used to stratify the records (U. S. Department of Agriculture 2009) (Fig. 1). These hydrologic units drain areas of markedly different topography and glacial history and were the smallest hierarchical drainage unit for which the data would support subdivision. The smallest subunit, the Wabash, was dropped from the analysis because it had only three records and a single species represented. Two indices of sample intensity for each HUC6 drainage were calculated by dividing the number of records and unique locations by the drainage area in km^2. Relationships between species richness, the number of unique locations, and drainage area and the SQRT of area

Table 2. Ohio stoneflies. Stream widths inhabiting and functional niche traits. Width 1=seep, 2=1–2 m, 3=3–10 m, 4=10–30 m, 5=30–60 m, 6=>60 m, 7=Lake Erie. Voltinism 1, 2 or 3 yr; development 1=fast, 2=slow seasonal. Diapause 1=present, 2=absent. Dispersal Season W=winter, Sp=spring, Su=summer. Feeding O=omnivore, P=predator, S=shredder. Female Mobility L=low, M=moderate, H=high. Nymphal Growth=months of growth, Respiration 1=no gills, 2=with gills. Size at maturity 1=<9 mm, 2=9–16 mm, 3=>16 mm. Emergence Synchrony 1=>1 mo., 2=<1 mo. Thermal preference 1=coldwater, 2=coolwater, 3=warmwater. Active hyperlinks are embedded LSIDs linking to species pages in the Plecoptera Species File website (DeWalt et al. 2012).

Species	**Species Niche Traits**											
	Stream Width	**Voltinism**	**Development**	**Diapause**	**Dispersal Season**	**Feeding**	**Fem. Mobility**	**Nymphal Growth**	**Respiration**	**Size at Maturity**	**Synchrony**	**Thermal Pref.**
Capniidae												
Allocapnia forbesi Frison	1–5	1	1	1	W	S	L	6	1	1	1	2
Allocapnia frisoni Ross & Ricker	2–3	1	1	1	W	S	L	6	1	1	1	2
Allocapnia granulata Claassen	3–6	1	1	1	W	S	L	6	1	1	1	3
Allocapnia illinoensis Frison	1–4	1	1	1	W	S	L	6	1	1	1	2
Allocapnia indianae Ricker	3–4	1	1	1	W	S	L	6	1	1	1	2
Allocapnia mystica Frison	2–4	1	1	1	W	S	L	6	1	1	1	2
Allocapnia nivicola (Fitch)	2–5	1	1	1	W	S	L	6	1	1	1	2
Allocapnia ohioensis Ross & Ricker	1–4	1	1	1	W	S	L	6	1	1	1	2
Allocapnia pechumani Ross & Ricker	3	1	1	1	W	S	L	6	1	1	1	2
*Allocapnia pygmaea (*Burmeister)	3	1	1	1	W	S	L	6	1	1	1	2
Allocapnia recta (Claassen)	2–5	1	1	1	W	S	L	6	1	1	1	2
Allocapnia rickeri Frison	2–5	1	1	1	W	S	L	6	1	1	1	2
Allocapnia smithi Ross & Ricker	2	1	1	1	W	S	L	6	1	1	1	2
Allocapnia vivipara (Claassen)	1–6	1	1	1	W	S	L	6	1	1	1	3
Allocapnia zola Ricker	3	1	1	1	W	S	L	6	1	1	1	2
Paracapnia angulata Hanson	1–4	1	2	2	Sp	S	M	11	1	1	2	1
Leuctridae												
Leuctra alexanderi Hanson	1–2	1	2	2	Su	S	M	11	1	1	1	2
Leuctra duplicata Claassen	3	1	2	2	Su	S	M	11	1	1	2	2
Leuctra ferruginea (Walker)	2–4	1	2	2	Su	S	M	11	1	1	2	2
Leuctra rickeri James	1–4	1	1	1	Sp	S	M	6	1	1	1	2
Leuctra sibleyi Claassen	2–4	1	1	1	Sp	S	M	6	1	1	1	2
Leuctra tenella Provancher	2	1	2	2	Su	S	M	11	1	1	2	2
Leuctra tenuis (Pictet)	2–4	1	1	2	Su	S	M	6	1	1	1	2
Paraleuctra sara (Claassen)	1–4	1	1	1	Sp	S	M	6	1	1	2	2
Zealeuctra claasseni (Frison)	2–3	1	1	1	W	S	M	6	1	1	1	2
Zealeuctra fraxina Ricker & Ross	2–3	1	1	1	W	S	M	6	1	1	1	2
Nemouridae												
Amphinemura delosa (Ricker)	1–5	1	1	1	Sp	S	M	6	2	1	1	2
Amphinemura nigritta (Provancher)	2–4	1	2	1	Su	S	M	9	2	1	1	1
Amphinemura varshava (Ricker)	1–6	1	1	1	Sp	S	M	6	2	1	1	2

Species	**Species Niche Traits**											
	Stream Width	**Voltinism**	**Development**	**Diapause**	**Dispersal Season**	**Feeding**	**Fem. Mobility**	**Nymphal Growth**	**Respiration**	**Size at Maturity**	**Synchrony**	**Thermal Pref.**
Nemouridae Cont.												
Nemoura trispinosa Claassen	1–3	1	2	2	Su	S	M	11	1	1	1	1
Ostrocerca albidipennis (Walker)	2–3	1	1	1	Sp	S	M	6	1	1	1	1
Ostrocerca truncata (Claassen)	2–3	1	1	1	Sp	S	M	6	1	1	1	1
Prostoia completa (Walker)	2–3	1	1	1	Sp	S	M	6	1	1	2	2
Prostoia similis (Hagen)	2–3	1	1	1	Sp	S	M	6	1	1	2	2
Soyedina vallicularia (Wu)	1–3	1	1	1	W	S	M	11	1	1	1	1
Taeniopterygidae												
Strophopteryx fasciata (Burmeister)	3–6	1	1	1	W	S	M	6	1	2	1	2
Taeniopteryx burksi Ricker & Ross	2–6	1	1	1	W	S	M	6	2	2	1	3
Taeniopteryx lita Frison	6	1	1	1	W	S	M	6	2	2	1	3
Taeniopteryx maura (Pictet)	2–5	1	1	1	W	S	M	6	2	2	1	3
Taeniopteryx metequi Ricker & Ross	3–5	1	1	1	W	S	M	6	2	2	1	2
Taeniopteryx nivalis Fitch	3–5	1	1	1	W	S	M	6	2	2	1	2
Taeniopteryx parvula Banks	3–5	1	1	1	W	S	M	6	2	2	1	2
Chloroperlidae												
Alloperla caudata Frison	2–4	1	1	1	Sp	P	M	6	1	1	2	2
Alloperla chloris Frison	2–4	1	2	2	Sp	P	M	11	1	1	2	1
Alloperla idei Ricker	3	1	2	2	Sp	P	M	11	1	1	2	1
Alloperla imbecilla (Say)	2–3	1	2	2	Sp	P	M	11	1	1	2	1
Alloperla neglecta Frison	3	1	2	2	Sp	P	M	11	1	1	2	1
Alloperla petasata Surdick	2–3	1	1	2	Sp	P	M	6	1	1	2	2
Alloperla usa Ricker	2–4	1	2	2	Sp	P	M	11	1	1	2	1
Haploperla brevis (Banks)	1–3	1	1	1	Sp	P	M	6	1	1	2	2
Sweltsa hoffmani Kondratieff & Kirchner	1–5	1	2	2	Sp	P	M	11	1	1	2	1
Sweltsa lateralis (Banks)	3	1	2	2	Sp	P	M	11	1	1	2	1
Perlidae												
Acroneuria abnormis (Newman)	3–6	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria carolinensis (Banks)	2–5	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria covelli Grubbs & Stark	5	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria evoluta Klapálek	4–6	2	2	2	Su	P	H	23	2	3	2	3
Acroneuria filicis Frison	2–6	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria frisoni Stark & Brown	2–7	1	2	2	Su	P	H	11	2	3	2	2
Acroneuria internata (Walker)	4–6	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria kosztarabi Kondratieff & Kirchner	3	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria lycorias (Newman)	2	2	2	2	Su	P	H	23	2	3	2	2
Acroneuria perplexa Frison	3–6	2	2	2	Su	P	H	23	2	3	2	3
Agnetina annulipes (Hagen)	4	2	2	2	Su	P	H	23	2	3	2	2
Agnetina capitata (Pictet)	2–5	2	2	2	Su	P	H	23	2	3	2	2
Agnetina flavescens (Walsh)	2–6	2	2	2	Su	P	H	23	2	3	2	2

Species	**Species Niche Traits**											
	Stream Width	**Voltinism**	**Development**	**Diapause**	**Dispersal Season**	**Feeding**	**Fem. Mobility**	**Nymphal Growth**	**Respiration**	**Size at Maturity**	**Synchrony**	**Thermal Pref.**
Perlidae Cont.												
Attaneuria ruralis (Hagen)	4	2	2	2	Su	P	H	23	2	3	2	3
Eccoptura xanthenes (Newman)	2	2	2	2	Su	P	H	23	2	3	1	1
Neoperla catharae Stark & Baumann	3–5	1	2	2	Su	P	H	11	2	2	2	2
Neoperla clymene (Newman)	3–5	1	2	2	Su	P	H	11	2	2	2	3
Neoperla coosa Stark & Smith	3–5	1	2	2	Su	P	H	11	2	2	2	2
Neoperla gaufini Stark & Baumann	3–6	1	2	2	Su	P	H	11	2	2	2	2
Neoperla mainensis Banks	4–7	1	2	2	Su	P	H	11	2	2	2	3
Neoperla occipitalis (Pictet)	3–6	1	2	2	Su	P	H	11	2	2	2	2
Neoperla robisoni Poulton & Stewart	3–5	1	2	2	Su	P	H	11	2	2	2	2
Neoperla stewarti Stark & Baumann	2–6	1	2	2	Su	P	H	11	2	2	2	2
Paragnetina media (Walker)	3–5	2	2	2	Su	P	H	23	2	3	2	2
Perlesta adena Stark	2–6	1	1	1	Su	P	H	4	2	2	2	2
Perlesta decipiens(Walsh)	3–6	1	1	1	Su	P	H	4	2	2	2	3
Perlesta golconda DeWalt & Stark	3	1	1	1	Su	P	H	4	2	2	2	3
Perlesta lagoi Stark	3–6	1	1	1	Su	P	H	4	2	2	2	3
Perlesta shubuta Stark	3–6	1	1	1	Su	P	H	4	2	2	2	2
Perlesta teaysia Kondratieff & Kirchner	2–5	1	1	1	Su	P	H	4	2	2	2	2
Perlesta xube Stark & Rhodes	4	1	1	1	Su	P	H	4	2	2	2	2
Perlesta sp. I–4	3-6	1	1	1	Su	P	H	4	2	2	2	2
Perlinella drymo (Newman)	3–5	1	1	1	Su	P	H	9	2	3	2	2
Perlinella ephyre (Newman)	3–6	1	1	1	Su	P	H	9	2	2	2	3
Perlodidae												
Clioperla clio (Newman)	1–5	1	1	1	Sp	P	H	9	1	2	2	2
Cultus decisus decisus (Walker)	2–3	1	2	2	Sp	P	H	11	1	2	2	1
Diploperla robusta Stark & Gaufin	1–5	1	1	1	Sp	P	M	6	1	2	2	2
Isoperla bilineata (Say)	3–6	1	1	1	Su	P	H	6	1	2	2	3
Isoperla burksi Frison	2	1	1	1	Sp	P	M	6	1	2	2	2
Isoperla decepta Frison	2–4	1	1	1	Sp	O	M	6	1	2	2	2
Isoperla dicala Frison	2	1	2	2	Su	P	H	11	1	2	2	2
Isoperla holochlora (Klapálek)	3	1	2	2	Su	P	H	11	1	2	2	1
Isoperla montana (Banks)	2–4	1	2	2	Sp	P	M	11	1	2	2	2
Isoperla nana (Walsh)	2–5	1	1	1	Sp	P	M	6	1	1	2	3
Isoperla signata (Banks)	2–4	1	2	2	Su	P	H	11	1	2	2	2
Isoperla transmarina (Newman)	2–4	1	2	2	Sp	P	H	11	1	2	2	2
Malirekus cf. *iroquois* Stark & Szczytko	3	1	2	2	Su	P	H	11	1	3	2	1
Peltoperlidae												
Peltoperla arcuata Needham	2–3	1	2	2	Su	S	H	11	2	2	2	1
Pteronarcyidae												
Pteronarcys cf. *biloba* Newman	3	3	1	1	Su	S	H	35	2	3	2	1
Pteronarcys dorsata (Say)	5–6	3	1	1	Su	S	H	35	2	3	2	2

were investigated using regression analysis as provided within the statistical program R version 2.14.0.

The NMDS analysis was conducted using PC–ORD Ver. 5 (PC–ORD 2011) to determine if there were relationships between HUC6 assemblages and a suite of environmental variables. A species presence/absence data matrix was constructed by HUC6 for the nine drainages. The matrix is available as "Appendix 4 spp. vs HUC6s". A second data set of 16 environmental variables was constructed that consisted of percentage pre-European settlement vegetation data (Ohio Department of Natural Resources 2003) and elevation, relief ratio, and slope variables (U. S. Geological Survey 2008). These data are included in Table 3.

Table 3. Nine Ohio HUC6 drainages, number of unique locations, species richness and 16 environmental variables used in NMDS analysis. Pre-European settlement vegetation is percentage cover. PEMM = Portage Escarpment Mesophytic Forest, Forest_UL =Upland forest, Forest_MX = Mixed deciduous/coniferous forest, WL_NW = nonwooded wetland, WL_W = wooded wetland, RR_Mean = Relief Ratio mean.

				% Pre-European Settlement Vegetation							Elevation (m)					Relief Ratio		
USGS HUC6s	**Sites**	**Species Richness**	**DRAIN_ km^{2}X1000**	**PEMM**	**PRAIRIE**	**FOREST_UL**	**FOREST_MX**	**SAVANNA**	**WL_NW**	**WL_W**	**ELEV_MEAN**	**ELEV_STD**	**ELEV_MAX**	**ELEV_MIN**	**ELEV_MEDIAN**	**RR_MEAN**	**RR_MEDIAN**	**SLOPE %**
WL_Erie	95	25	23.31	0.0	2.3	59.3	0.7	2.0	1.6	34.1	239	35.8	411	147	233	0.35	0.33	0.8
SE_LErie	133	65	8.25	1.2	0.1	68.8	24.0	0.0	1.0	4.9	285	53.9	420	173	285	0.45	0.45	2.2
UOH_ Bvr	50	44	8.62	0.0	0.0	80.2	17.2	0.0	0.2	2.4	330	38.6	436	182	336	0.58	0.61	6.2
UOH_ LKan	80	48	7.93	0.0	0.2	80.7	15.9	0.0	0.3	2.9	263	45.8	433	151	261	0.40	0.39	10.3
Musk	116	56	20.85	0.0	0.0	76.2	20.6	0.0	0.4	2.8	316	43.0	460	174	317	0.50	0.50	6.8
Scioto	201	71	16.87	0.0	3.9	69.9	12.1	0.0	0.3	13.7	283	43.2	454	142	289	0.45	0.47	3.9
GrMiami	109	34	10.70	0.0	1.3	79.4	5.1	0.0	0.4	13.8	299	44.7	469	138	306	0.49	0.51	1.9
MOH_ Rac	39	35	5.06	0.0	0.0	84.9	10.0	0.0	0.0	5.1	233	34.1	363	136	234	0.43	0.43	9.8
MOH_ LMia	119	57	9.37	0.0	1.8	60.8	16.4	0.0	0.0	21.0	269	45.5	404	131	275	0.50	0.53	4.6

Results

Species Richness and Community Composition

A total of 4,051 database records accounting for 32,487 specimens were accumulated for this project (Table 1). The museum that contributed the most records was the Bean Museum at Brigham Young University, followed by the INHS Insect Collection. Cumulatively, these records produced an Ohio stonefly assemblage of at least 102 species (Table 2) from 942 unique locations (Fig. 1, Appendix 2).

Species richness estimators predicted slightly higher values (Fig. 3). The Chao2 estimator predicted 105.6 species with 95% confidence intervals (CI) ranging from 102.8 to 119.0 species. Another estimator, ICE Mean, produced a mean value of 106.4 (EstimateS does not provide CIs for this estimator). Many rare species were found. Singletons and doubletons accounted for 17.6% of all species (Fig. 4). Approximately 75% of all species were collected at fewer than 20 locations, while only

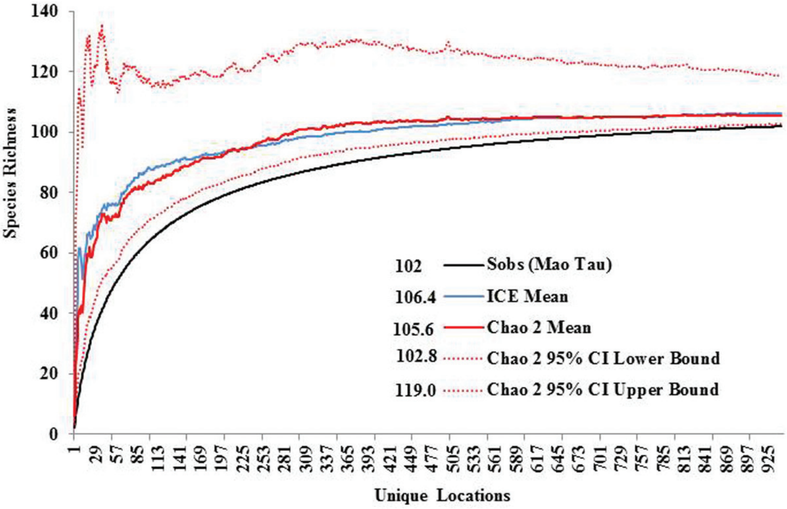

Figure 3. Ohio Plecoptera species richness, actual vs. predicted.

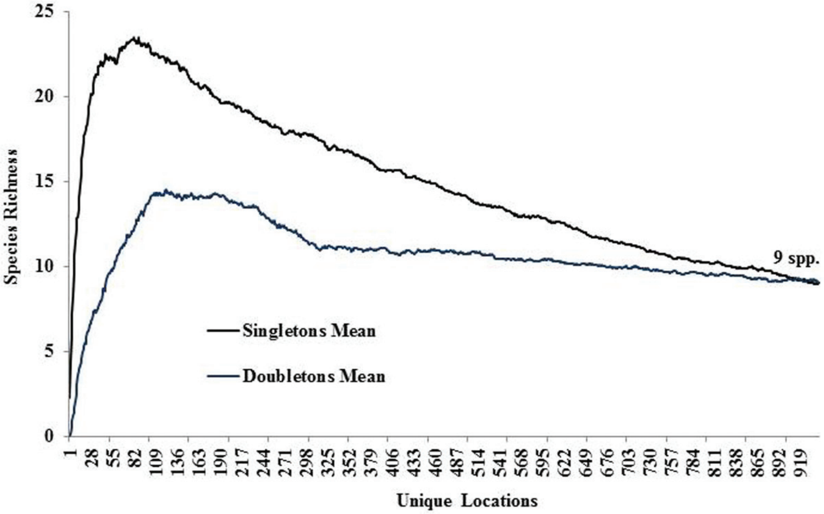

Figure 4. Singleton and doubleton species richness.

three species were collected from over 100 locations: *Allocapnia vivipara* (Claassen) (223 sites), *Perlesta* cf. *lagoi* Stark (161 sites), and *Acroneuria frisoni* Stark & Brown (115 sites) (Fig. 5).

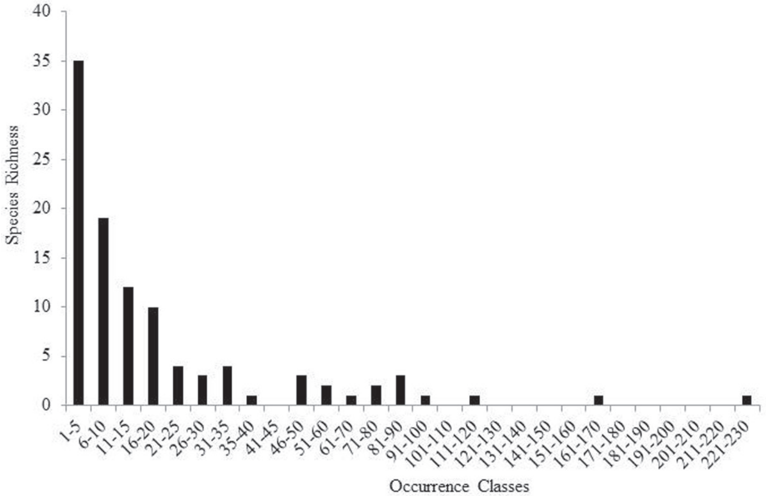

Figure 5. Species richness of Ohio Plecoptera in 5 increment occurrence classes.

Assemblage Composition

The stonefly fauna of Ohio was represented by all nine families known to inhabit the North American continent (Fig. 6). Perlidae contributed the greatest number species (32.4%). Capniidae, one of the winter stonefly families, contributed 15.7% of all species found. Four other families contributed between 8.8% and 12.7% of the fauna. Roach stoneflies, Peltoperlidae, contributed a single species, *Peltoperla arcuata* Needham.

Stream size is often an important determinant of stonefly communities. This dataset demonstrated that most species inhabited a range of stream sizes (Table 2). There were several species relegated to small streams. Five species occurred only in streams that were 1 to 2 m across: *Allocapnia smithi* Ross & Ricker, *Leuctra tenella* Provancher, *Eccoptura xanthenes* (Newman), and *Isoperla burksi* Frison. Several others inhabited streams 3 to 10 m across: *A. pechumani* Ross & Ricker, *A. zola* Ricker, *L. duplicata* Claassen, *Acroneuria kosztarabi* Kondratieff & Kirchner, *Peltoperla arcuata* Needham, and *Malirekus* cf. i*roquois* Stark & Szczytko. While some species were most frequently collected from large rivers, they could be taken in somewhat smaller streams as well. Only two species were confirmed to inhabit Lake Erie, from several locations around the Bass Islands: both perlids, *A. frisoni* and *Neoperla mainensis* Banks. Both species have been extirpated from Lake Erie, but *A. frisoni* is frequently collected throughout all but the northwestern corner of the state.

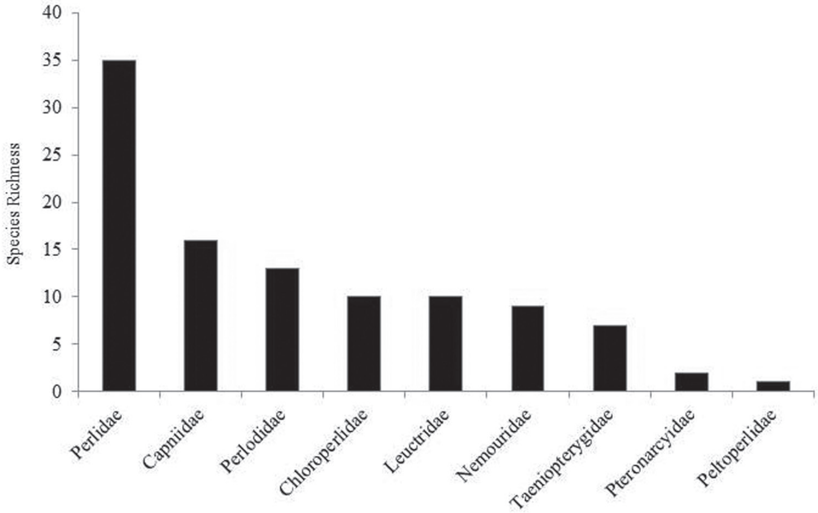

Figure 6. Species richness of Ohio Plecoptera families.

Distribution of Species Traits

The vast majority of stonefly species inhabiting Ohio have single year life cycles; only 17 (16.7%) had multiyear life cycles (Table 4). Species with fast–seasonal cycles, those with nymphal growth periods that lasted 4-9 months, are slightly more frequently collected than slow seasonal (growth 11, 23, 35 mo. = direct development) species. Species with egg or nymphal diapause were also more frequently collected in Ohio than were non–diapausing species. Most species dispersed in the summer, a trait state that was not surprising given the number of perlid species found. Ohio did have quite a large proportion of so called "winter stoneflies", species that emerge in winter that belong to the families Capniidae and Taeniopterygidae.

The number of months of nymphal growth had the largest number of trait states of all species trait categories. There were 9 species with an exceedingly short growth period of four months. These included all *Perlesta* species, a genus where females lay eggs in June and July and the eggs diapause until March. The most frequent growth period was 6 months, accounting for 40.8% of all species. Only a few species exhibited a nine month growing period, *Perlinella*, *Clioperla* clio (Newman), and *Amphinemura nigritta* (Provancher), while a total of 31 exhibited 11 month growth periods. Growth periods lasting 23 (*Acroneuria, Agnetina, Attaneuria, Paragnetina*) and 35 (*Pteronarcys*) mos. were also present.

More research on the feeding of Plecoptera nymphs and adults is necessary to effectively use functional feeding group designations for ecological research. The study of gut enzymatic activity (Tierno de Figueroa et al. 2011) and use of stable isotopes to determine nutrient sources (Miyasaka and Genkai-Kato 2009, Reynolds et al. 1997) has demonstrated how little we understand about the feeding of stoneflies. The func-

Table 4. Species traits distributions for the Ohio stonefly assemblage. Traits from Table 2.

Volt.		**Devel.**		**Diap.**		**Dispers.**		**Feed.**		**Mobil.**		**Grow.**		**Respir.**		**Size (mm)**		**Synch. (mo.)**		**Therm.**	
1	85	1	57	1	56	W	25	O	1	L	15	4	8	1	56	<9	46	>1	36	1	19
2	15	2	45	2	46	Sp	28	P	56	M	42	6	42	2	46	9–16	35	<1	66	2	67
3	2					Su	49	S	45	H	45	9	4			>16	21			3	16
												11	31								
												23	15								
												35	2								

tional feeding group designations presented herein must be considered tentative, but should be useful to characterize the distribution of feeding groups within Ohio. Predators were the most frequent functional feeding group found among Ohio species, a function of the dominance of perlids once again. The next largest feeding group was the shredders of tree leaves. One species has been listed as an omnivore, *Isoperla decepta* Frison (Perlodidae), since its lacinia have apical paired, chisel-like teeth that may indicate that scraping of periphyton is an option at least during part of its nymphal life. That it is a perlodid suggests that it might also eat animal matter. It is probable that when detailed enzymatic or stable isotope studies are conducted on stoneflies, many species will be viewed as omnivorous, at least during part of their nymphal growth.

Female mobility is an important trait that confers ability to colonize and recolonize after local extinction. The vast majority of species exhibited medium to high female mobility. Low female dispersal ability was exhibited by 14.6% of Ohio species. Low mobility is a complex character state that is exhibited mostly by winter emerging species that emigrate by crawling, skating (wings held up to breeze and skating on tarsi), or floating on logs or ice floes.

The presence or absence of gills is often thought of as indicative of a species' ability to tolerate warmer waters and lower oxygen concentrations. No formal analysis has been conducted of the association of gilled stoneflies with water temperature preference, but informal studies in Europe indicate that many gilled species inhabit mountainous areas with cold and cool water temperatures (M. Tierno de Figueroa pers. comm.). In eastern North America there are over 50 species of perlids (*Attaneuria, Perlesta, Perlinella, Neoperla, Acroneuria, Agnetina,* and *Paragnetina)* that tolerate warmer streams and often dominate the stonefly assemblage at low elevation.

The majority of Ohio species (54.9%) did not have gills (some Perlodidae with submental gills were counted as gill-less). Most of these species lived in cool and coldwater streams or lived in streams as nymphs only during the colder times of the year (e.g., they have diapause that restricts nymphs to winter or spring season). A total of 45.1% of species used gills. The vast majority of these were species in the family Perlidae.

Size at maturity is a trait that has direct bearing on risk to survival. Larger species are usually longer lived and exposed to risks for a longer period of time and may make more attractive prey items than some smaller species. Small species (<9 mm total length) were much more frequent in the list than large species (>16 mm). Smaller species are more likely to have short growth periods and diapause. They are also more likely to have fast cycles and disperse in the winter and spring than larger species.

Species with synchronous (<1 month) emergence periods were more frequent in Ohio than asynchronous (>1 month) species. Winter emerging species tend to have less synchronous emergence periods due to fluctuating winter temperatures from freezing and thawing. Those that emerge in spring and summer have sharper seasonal cues that lead to nymphal development being more synchronous, leading to emergence of adults over a shorter period of time. Spring and summer emerging species were three times more frequent in Ohio than winter-emerging ones; hence, the great disparity in synchronous over asynchronous emergence is easily understood.

Thermal requirements are not well understood in stoneflies or other aquatic insects and the terms coldwater, coolwater, and warmwater are relative when it comes to defining a temperature requirement. Trait state assignment here is based more on professional experience than for any other set of traits. Coldwater species contribute only 18.6% to the total number of species found in OH. Most of Ohio is or was heavily wooded, a feature usually related to coolwater conditions. This is by far the most frequent thermal tolerance state for stoneflies in Ohio. Another 15.7% of species can truly be categorized as warmwater species. This would include several perlid species.

Does drainage affiliation affect assemblage composition and species richness? Two measures of sample intensity across the nine HUC6 drainages demonstrated that three HUC6s were most heavily sampled: Southern and Eastern Lake Erie, Scioto, and Middle Ohio and Little Miami drainages (Fig. 7A). The two largest drainages, Western Lake Erie and Muskingum appeared to be somewhat under-sampled compared to other drainages. Across all drainages, species richness was significantly related to the number of unique locations sampled (R^2=0.42, p=0.03, n=9, Fig. 7B). Western Lake Erie and Great Miami drainages had lower than predicted species richness. These are the western-most drainages in the state with a landscape composed of flatter till and lake plains, possibly accounting for lower species richness. There was no significant relationship between species richness and drainage area (R^2=0.0, p=0.87, n=9) or the square root of drainage area (R^2=0.0, p=0.97, n=9).

Randomization tests in the NMDS analysis recommended a three dimensional solution. An overall stress value for the three dimensional analysis was low at 1.53. A plot of Axis 1 vs Axis 3 separated the communities of Ohio stoneflies best (Fig. 8). The western–most HUC6 drainages (Western Lake Erie and Great Miami) were separated from all other assemblages by being strongly associated with wetland and savanna pre-European settlement land covers. There was a lesser association with large total area as well. These two drainages supported the lowest species richness. Centrally located in the plot were five HUC6 communities that associated with low percentage wetland and savanna coverage and with smaller drainage sizes. These drainages had much richer stonefly communities and an association with upland hardwoods. Two other HUC6 assemblages (Upper Ohio Little Kanawha and Middle Ohio Raccoon) were separated from the others by being associated with a high percentage of mixed deciduous/coniferous forest and high percentage slope.

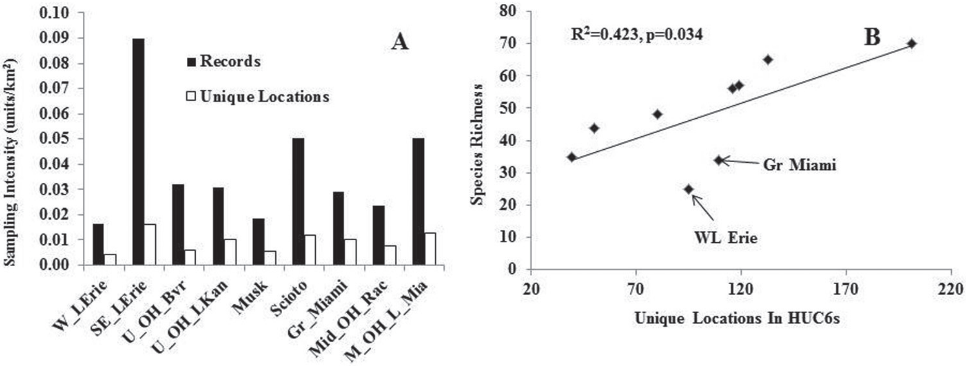

Figure 7. A–B Sampling intensity, drainage area, unique locations, and species richness relationships for HUC6 drainages **A** Sampling intensity for HUC6 drainages **B** Species richness vs. number of unique locations in HUC6 drainage areas.

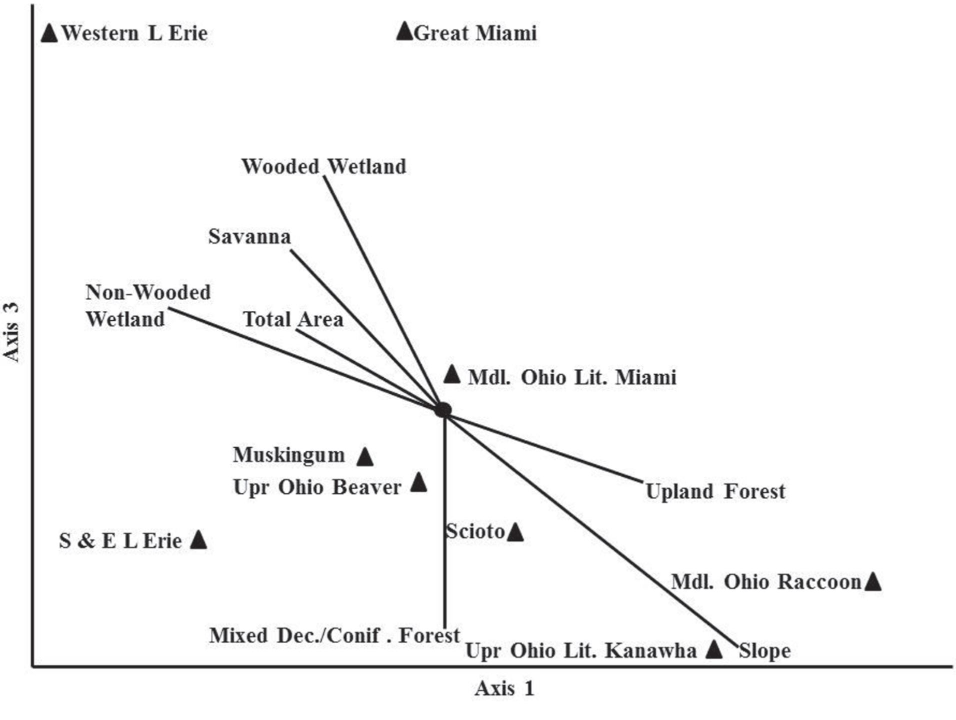

Figure 8. Non–parametric Multi–Dimensional Scaling of Ohio Plecoptera assemblages associated with HUC6 drainages. Axis 1 vs Axis 3.

Discussion

Comparison to assemblages found in nearby states/provinces

Ohio is a state that has its eastern flank in the Allegheny Plateau, an extension of the Appalachian Mountains. Ohio's western flank is mostly till plain resulting from the Wisconsinan glaciation. The stonefly fauna found in the state is a mixture of species requiring cooler waters and deep forest and those that have evolved with warmwater streams and even intermittency of flow. The number of species occurring in Ohio is indicative of being between these two extremes. Ohio supports at least 102 species, maybe as high as 119 (e.g., Chao 2 upper 95 percentile) (Fig. 4). Pennsylvania to the east supports 39.2% more species than Ohio, with West Virginia having 36.3% more species. It appears that a continuous drop of species occurs westward and northward from Ohio (Fig. 9). The northward decline of species occurs as species usually inhabiting unglaciated terrain drop out. Sørensen's Index of Similarity suggested that Kentucky and Indiana assemblages have the greatest similarity with the Ohio assemblage, rather than the more mountainous states of Pennsylvania and West Virginia (Table 5). The lowest similarities were observed with more recently glaciated Michigan, Ontario, and Wisconsin.

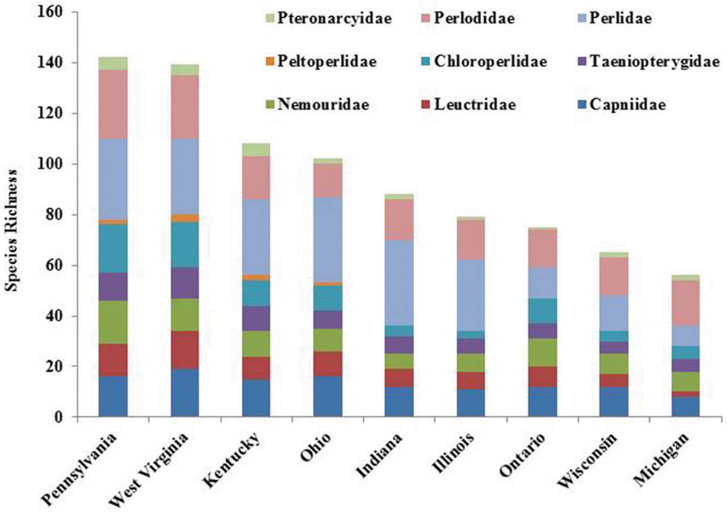

Figure 9. Comparison of Ohio Plecoptera assemblage with Midwest states/provinces.

Table 5. Sørensen Index of Similarity between Plecoptera assemblages for nearby states/provinces in relation to Ohio. Codes are IL=Illinois, IN=Indiana, MI=Michigan, OH=Ohio, ON=Ontario, WI=Wisconsin, PA=Pennsylsvania, WV=West Virginia, and KY=Kentucky.

	IL	IN	MI	OH	ON	WI	PA	WV	KY
IL	0								
IN	0.814	0							
MI	0.444	0.417	0						
OH	0.659	0.743	0.415	0					
ON	0.416	0.429	0.656	0.551	0				
WI	0.569	0.523	0.744	0.548	0.686	0			
PA	0.425	0.470	0.394	0.629	0.535	0.512	0		
WV	0.440	0.485	0.349	0.628	0.467	0.461	0.826	0	
KY	0.588	0.684	0.354	0.749	0.437	0.474	0.648	0.721	0

Rare species

Ohio had 18 species that were collected from just one or two locations, 17.6% of all species found. A discussion for a limited number of these species is presented below to identify for state, federal, and non–profit conservation organizations that these species, and the streams in which they reside, should be considered for protected status. Some streams are already in the public trust.

Allocapnia indianae Ricker. A large series was collected by W. E. Ricker (1952) on 19 March 1950 from three locations in Scioto Co.: W Portsmouth, Turkey Creek, 38.69690, –83.10076; W. Portsmouth, road 25, Odell Creek, 38.70286, –83.11518; Rd. 125, 9 mi. E Blue Creek, Turkey Creek, 38.727194, –83.17265. The species in known from scattered locations in unglaciated areas of Indiana, Kentucky, and Ohio, but has also been collected in the Finger Lakes region of New York (Ross and Ricker 1971).

Allocapnia smithi Ross & Ricker. This species was taken from a single location in Warren Co., 10 km ESE Lebanon, Randall Run, Fort Ancient State Memorial, 39.40951, –84.09039, 12 February 1966, H. B. Cunningham. This species is known from scattered locations in unglaciated sections of Alabama, Illinois, Indiana, Kentucky, and Ohio (DeWalt and Grubbs 2011, Ross and Ricker 1971).

Leuctra duplicata Claassen. This species was taken from two small streams adjacent to each other in Ashtabula Co.: 4.5 km NNW Hartsgrove at Callahan Rd., Crooked Creek, 41.64210, –80.97250, 3 June 1997, R. W. Baumann & B. C. Kondratieff, 7 males, 9 females; same location, 2 June 1989, R. W. Baumann & R. F. Kirchner, 2 M; Callahan Road, spring fed tributary Crooked Creek, 41.64245, –80.97374, 2 June 1989, R. W. Baumann & R. F. Kirchner, 42 males, 28 females. Crooked Creek is a darkly stained, 3–m wide stream that holds other coolwater species such as *Acroneuria carolinensis* (Banks)*, Isoperla* cf. *montana* (Banks)*, Soyedina vallicularia* (Wu)*, Paracapnia angulata* Hanson*, Allocapnia rickeri* Frison*,* and *Taeniopteryx metequi* Ricker & Ross. This species is known from much of northeastern North America, but Ohio is the furthest west it has been collected (DeWalt et al. 2011).

Alloperla idei Ricker. This species is represented by two records in the Allegheny Plateau region of SE OH. Lawrence Co., 17 km SSE Oak Hill, Buffalo Creek, Wayne National Forest, 38.74598, –82.54445, 27 May 2010, S. A. Grubbs, 3 males, 9 Females; Pickaway Co. Laurelville, Tributary Laurel Run, 39.47390, –82.74263. 23 May 1953, [A. R. Gaufin], 2 males. This species occurs throughout much of eastern Canada and the eastern USA (Baumann and Kondratieff 2009, DeWalt et al. 2011).

Alloperla neglecta Frison. This species was collected as a single adult male from Lake Co., at Paine Road, Leroy Township, Paine Creek, Paine Falls Metropolitan Park, 41.71669, –81.14356, 31 May 1975, M. K. Tkac. Tkac (1979) considered this a relict population in a pristine stream. The nearest known populations of this species are in North Carolina, Tennessee, and Virginia (DeWalt et al. 2011).

Sweltsa lateralis (Banks). Several adults were collected from Mahoning Co., Lowellville, Grays Run, 41.04353, –80.53957, May 1985, D. W. Fishbeck. This Gray's

Run was recorded as supporting several other species of Appalachian Mountains or coldwater habitat.

Peltoperla arcuata Needham. This species was once thought to be exceedingly rare in Ohio, but it has been located in four counties now in 1 to 2–m wide coldwater, ravine streams, usually associated with mixed deciduous and coniferous (hemlock and white pine) forest. This is the only representative of the family Peltoperlidae to inhabit Ohio. Loss of this species would remove an entire family of stoneflies from the state. Locations include: Ashland Co., Tributary to Hog Hollow Creek, Big Lyon Falls Creek, Little Lyon Falls Creek, Tributary Clear Fork Mohican River (Hemlock Grove CG), all in Mohican State Park; Knox Co., Tributary Mohican River at Greer; Mahoning Co., Gray's Run at Lowellville; Muskingum Co., Seep Tributary Wills Creek. The species is distributed over several eastern states and the Canadian province of Quebec (DeWalt et al. 2011).

Isoperla burksi Frison. Three nymphs were collected from Scioto Co., Mackletree Run, 12 km SSW West Portsmouth, Shawnee State Forest. 38.7236, –83.1821, 14 April 2006, R. E. DeWalt, S. K. Ferguson, R. F. Kirchner. This species has been collected from the Ozark Mountains to New Jersey, mostly in unglaciated habitats. Mackletree Run is a stream that typifies semi–permanent streams in unglaciated southern Ohio.

Malirekus cf. *iroquois* Stark & Szczytko. We have seen two nymphs from a single location in Monroe Co., Tributary Stillhouse Run, 39.78146, –80.85288. M. Leuhrs. Fishbeck (1987) reported *M. hastatus* (Banks) from Gray's Run. However, Stark and Szczytko (1988) described *M. iroquois* from nearby states after Fishbeck's work, so the identification of these specimens is in doubt. Nonetheless, this is a second location for *Malirekus* in Ohio and an additional Appalachian species in Grays Run.

Pteronarcys cf. *biloba* Newman. Nymphs with lateral extensions of the abdominal terga have been known from Ohio since Tkac's (1979) dissertation, but no adults have been collected and the record was not published. Bolton (2010) published the first Ohio record for this Appalachian species, but from a second location. Ashtabula Co., Indian Creek, Montgomery Road, RM 1.3, 41.5644, –80.9328, 11 September 2007, M. J. Bolton, nymph; Lake Co., Kirtland Hills at Sperry Road, Pierson's Creek, 41.62818, –81.31494, 5/11/1978, M. K. Tkac, nymph; same location, 5/20/1978, M. K. Tkac, nymph. These Ohio records are the furthest west for the species; it is found throughout northeastern North America as far south as northern Georgia (Nelson 1971).

Streams with diverse assemblages

Several streams across the state are exceedingly rich in species and have been well sampled. A tributary of the East Branch of the Chagrin River in Stebbins Gulch of Holden Arboretum (Geauga Co.) produced 28 species including several coldwater species. A tributary of the East Fork Queer Creek at Ash Cave (Hocking Co.) has produced 23 species. The Olentangy River near Columbus (Franklin Co.) has produced 17 species historically. Upstream of the city near Highbank Metropark a diverse assemblage still persists, although it may not hold the full complement of species once found in the river. The Clear Fork of the Mohican River within the ravine area of Mohican State Park (Ashland Co.) has produced 14 species and probably still supports most of them. Big Lyons Falls Creek, also in Mohican State Park; a tributary of the North Fork Little Beaver River, 5 km S Negley, in Columbiana Co.; and Mill Creek at Doty Road (Lake Co.) all produced 13 species. Gray's Run at Lowellville (Mahoning Co.) produced a total of 12 species, many of which were Appalachian coldwater species. Most of these locations are protected by public or private means. These, and several others too numerous to list here, are important for protecting the lotic diversity of aquatic organisms in the Ohio.

HUC6 Drainages

HUC6 drainages explain some of the variation in stonefly communities across Ohio (Fig. 8). However, the stress value was relatively low for this analysis, suggesting that there may be better stratification systems and variables to explain Ohio species richness patterns. Additional classification systems that could be tested in future analyses include U. S. Environmental Protection Agency level III ecoregions and the Nature Conservancy's Ecological Drainage Units.

The data suggested that smaller drainages of the eastern and southern part of the state followed a pattern of increasing richness with drainage area, but that the largest drainage did not (Fig. 7B). Western Lake Erie was under-sampled in relation to its area (Fig. 7A, B). However, this is not to say that the species richness in the drainage was suspiciously low. The Western Lake Erie assemblage was defined by wetland categories and large drainage area (Fig. 8). This basin has low topographic relief and fine substrates left behind by large glacial lakes. The low species richness there was most likely a function of the flatter landscape, low current velocity, and finer substrates. The Great Miami drainage just to the south of Western Lake Erie also had relatively low species richness. It too was defined by similar environmental conditions as Western Lake Erie, however, its southern end contains one coldwater stream (Mad River) and fast flowing reaches, which enhanced its species richness over that of Western Lake Erie. Indeed, the two drainages had a 61% Sørensen quotient of similarity. The Great Miami basin supported such cool- and coldwater species as *Agnetina capitata* (Pictet), *Paragnetina media* (Walker), *Leuctra tenuis* (Pictet), *Nemoura trispinosa* Claassen, and *Soyedina vallicularia* (Wu). Several species that require faster flowing, wooded streams were also found in the Great Miami and not the Western Lake Erie drainage.

Some of the most species rich HUC6 assemblages were located in a band of upland forest and higher gradient streams that straddled drift plain and unglaciated terrain from Cincinnati to Ashtabula. These assemblages were dominated by widespread species that typically inhabit cool and warmwater streams. There was also a small component of coldwater Appalachian fauna including species of *Ostrocerca*, *Amphinemura nigritta*, *Cultus d. decisus*, *Malirekus* cf. *iroquois*, *Pteronarcys* cf. *biloba*, *A. neglecta*, *Alloperla lateralis*, *Alloperla idei*, and *Allocapnia pechumani*, among others. These species have found coldwater habitat in ravine streams of Southern and Eastern Lake Erie, Upper Ohio and Beaver, Scioto, and Muskingum drainages.

Two other drainages, the Upper Ohio Little Kanawha and the Middle Ohio Raccoon, had assemblages that were defined by mixed deciduous and coniferous forests and higher slope values in southern, unglaciated Ohio. These are relatively small drainages with short streams, many of which become intermittent in the summer. Consequently, many species with egg or nymphal diapause are found here. This trend also occurs in southern Illinois and Indiana (DeWalt et al. 2005, DeWalt and Grubbs 2011, Webb 2002).

Changes in the fauna

The Plecoptera assemblage presented herein is largely of pre-European settlement nature from records spanning the late 1880s to present. Some changes are likely to have occurred in Ohio, just as they did in IL (DeWalt et al. 2005). Additional targeted sampling would be required to assess changes in detail. However, some changes are readily apparent, mostly the loss of the large perlid species that lived in larger rivers and had 11 or 23 month nymphal growth.

Acroneuria abnormis (Newman). Once found in moderately large to large rivers throughout the state, it is now only known from recent records in the Grand River (Lake Co.), Clear Fork Mohican River (Ashland Co.), and Ohio River (Clermont Co.).

Acroneuria frisoni Stark & Baumann. This species was found at 115 unique locations across the state, with many being from recent years. Many historical records of adults and nymphs were found for the Bass Islands in the Western Basin of Lake Erie. Unfortunately, of the 94 records from the islands, the most recent was for 1961. It has been extirpated from Lake Erie.

Acroneuria filicis Frison. It once inhabited several moderately sized drainages in the eastern half of the state. The only recent records are from the Grand River (Lake Co.), West Fork River (Brown Co.), and Ohio Brush Creek (Scioto Co.).

Acroneuria perplexa Frison. This species has been lost from moderately large and large rivers in the eastern half of Ohio. No records are available for over 50 years. It is probably extirpated from Ohio.

Acroneuria evoluta Klapálek: There is a single historical record from 1936 from Black Lick Creek near Columbus (Franklin Co.). It is probably extirpated from Ohio.

Attaneuria ruralis (Hagen). A male and female of this large species were collected from Columbus (Franklin Co.), either from the Olentangy or Scioto rivers, in 1925. It is probably extirpated from Ohio.

Neoperla mainensis Banks. This species was historically collected from Columbus (Franklin Co.), either from the Scioto or Olentangy rivers; the Clear Fork of the Mohican (Ashland Co.), and South Bass Island (Ottawa Co.) area of Lake Erie. It is probably extirpated from the state with none of the 38 records being more recent than 1922.

Conclusions

A large dataset from 18 regional museums, new collecting, and trusted literature sources demonstrated that the Plecoptera assemblage of Ohio is rich with at least 102 species. All nine North American families were represented with Perlidae contributing 33% of all species (Fig. 6).

Ohio species are mostly univoltine-fast with egg or nymphal diapause and the largest proportion of them are summer emerging (Table 4). Nymphs are predominantly predatory, but a large number of species that shred conditioned leaves and wood were also present. The majority of species inhabited cool, forest-covered streams. The state has significant Appalachian elements, mostly within the ravine streams of central and eastern drainages associated with upland forests and mixed deciduous and coniferous forest habitats (Fig. 8). Despite this, the fauna of Ohio is most closely aligned with Indiana and Kentucky assemblages (Table 5).

It appears that Ohio has been well sampled; species estimators (Chao2 and ICE Mean) suggested that 3 or 4 more species could be found. Given that neighboring Pennsylvania and West Virginia have 142 and 139 species, respectively, it is likely that species shared with them will eventually be found in Ohio (Fig. 9). It is highly probably that these additional species will be located in ravine streams in eastern and southern Ohio.

A great number of species in Ohio were rare, being known from only 1–5 locations (Figs. 4 and 5). Many of these are candidates for protection, as are several stream reaches that support high numbers of species or assemblages that are rare. In addition, it appears that several species have experienced local or statewide extirpations. These have mostly been of univoltine and semivoltine species whose eggs hatch directly. This is consistent with losses reported for IL (DeWalt et al. 2005). Conservation organizations working in Ohio may download these data directly supplemental files.

This paper lays a foundation for planned future work, including natural range modeling of species within the larger framework of the Midwest USA and Canada. This will allow our research team to reconstruct pre-European settlement ranges for most species in the region. We are also focusing considerable effort to use these baseline distributions against which to measure climate related changes in distribution by modifying climate variables in light of predicted CO_2 emissions scenarios.

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Appendices

Raw data and data matrices. (doi: 10.3897/zookeys.178.2616.app1) File format: MS Excel spreadsheet (xls).

Explanation note: Appendices include an Excel spreadsheet with all data used in analyses for this article: "Appendix1 Raw Specimen Data", "Glossary Appendix 1", "Appendix 2 OH Unique Locations", "Appendix 3 spp. Location Matrix", and "Appendix 4 spp. vs HUC6s".

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RESEARCH ARTICLE

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The soil mite genus *Conchogneta* (Acari, Oribatida, Autognetidae), with new findings from Mongolia

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Abstract

This work deals with taxonomy, geographical distribution as well as known ecology of oribatid mites of the genus *Conchogneta* Grandjean, 1963 in the world. The majority of species belonging to this genus is known to be widely distributed in Europe, but only three of them are found in other areas of the northern hemisphere. Most species of *Conchogneta* are inhabitants of litter of various types of forestas, terricolous and epiphytic bryophytes, epiphytic lichens, and soil of steppe, river valleys, moor, oligotrophic bogs, floodland assemblages etc. A new species, *Conchogneta glabrisensillata* **sp. n.** is described, and another species, *C. traegardhi* (Forsslund, 1947) is redescribed from the northern and western parts of Mongolia, respectively. *Conchogneta* is recorded for the first time for the fauna of Mongolia. The species status of *C. dalecarlica* (Forsslund, 1947) is discussed. Species descriptions are accompanied with detailed illustrations. Furthermore, a key is provided for the identification of adults of the known species of *Conchogneta* in the world.

Keywords

Oribatida, *Conchogneta*, biogeography, habitat ecology, new species, Mongolia

Introduction

The oribatid mite genus *Conchogneta* is one of seven genera in the family Autognetidae, which was erected by Grandjean (1963) with the type species, *Autogneta dalecarlica* Forsslund, 1947. Currently, the genus comprises seven nominal species and one subspecies, the majority of which have restricted distributions in the Palaearctic region, especially in Europe. Two eastern European species, *Conchogneta vasiliorum* Mahunka, 2006 and *C. weigmanni* Mahunka, 2007 are known until today only from Romania; another species, *C. inundata* (Winkler, 1957) is reported from the Czech Republic; *C. willmanni herzegowinensis* (Willmann, 1941) is known from Bosnia and Herzegovina. Three other species, *C. traegardhi* (Forsslund, 1947), *C. willmanni* (Dyrdowska, 1929) and *C. dalecarlica* (Forsslund, 1947) have rather wide distributions in the Palaearctic or even Holarctic region, and all these three species were recorded in Europe and Asia; *C. traegardhi* (Forsslund, 1947) was also found in North America. *Conchogneta iranica* Akrami, 2008 is the single species which is only known from Asia (Dyrdowska 1929, Willmann 1941, Forsslund 1947, Winkler 1957, Grandjean 1963, Woas 1986; Marshall et al. 1987, Mahunka 2006, 2007, Akrami 2008, Toluk and Ayyildiz 2009).

The genus *Conchogneta* is unique among other genera of Autognetidae in the combination of following characters: rostrum with deep medial incision; prodorsal costulae long, mostly medially positioned close to each other, but rarely distantly placed laterally from each other; sensilli narrow, setiform or with dilated head; anterior part of notogaster without crista; tibia I with large dorso-distal tubercle overhanging tarsus I.

The immatures of *Conchogneta* are apheredermous, which means nymphs (and adults) do not retain scalps, unideficient, and have setae *d* on tibiae and genua of legs when respective solenidia exist. However, the morphology of immatures of most *Conchogneta* species is poorly known, and only two of them, such as *C. dalecarlica* and *C. traegardhi* are studied in terms of juvenile morphology and patterns of their postembryonic development (Grandjean 1963, Ermilov and Łochynska 2009, Ermilov 2011).

The aim of this work is to describe an unknown species, *Conchogneta glabrisensillata* sp. n., and redescribe another recently collected species, *C. traegardhi* (Forsslund, 1947) from northern and western Mongolia, respectively. The latter species is recorded for the first time in Mongolia. A review of the composition of genus *Conchogneta* with remarks on the biogeography and habitat ecology of its members, and a wold-wide identification key to *Conchogneta* are additionally provided. The taxonomic status of *C. dalecarlica* is discussed, which was argued previously in different literature. The study of oribatid mite diversity in Mongolia is the subject of ongoing research as part of the biodiversity assessments in various habitats of the country with emphasizes of the effects of climate change and influence of pastoral livestock grazing.

Material and methods

All materials used in this study were collected by the author with assistance of some of his graduate students and specimens were mounted in temporary slides to view the anterior, lateral and posterior aspects, and then preserved in alcohol. All examined materials and data on their localities are given in the respective 'material examined' section. Species studied here are represented as adults.

Specimens were cleared in lactic acid, and a differential interference contrast microscope was used for investigation in transmitted light. Line drawings were made using a camera lucida attached to the compound microscope. Micrographs were taken using a digital camera (Olympus Altra 20) attached to the microscope with single shot.

The morphological terminology used below is mostly that developed over many years by Grandjean (1960a, b, 1963), and also that by Lions (1975), Norton and Behan-Pelletier (2009). All measurements are given as a range, with the mean in parentheses. Body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate, to avoid discrepancies caused by different degrees of notogastral distension. Notogastral length was also measured in lateral aspect (when the dorsosejugal groove is discernable), from the anterior to the posterior edge; notogastral width refers to the maximum width in dorsal aspect. Setal formulas of the legs are given as numbers per segment for appendages (from trochanter to tarsus) and as number per podosomal segment (I-IV) for epimeres. Most species of Autognetidae show the same structure and setation of legs, palps and chelicerae. Therefore, in this work I made detailed descriptions and illustrations of the chelicera and the palp only for one of the studied species.

Results

Conchogneta glabrisensillata sp. n.

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http://species-id.net/wiki/Conchogneta_glabrisensillata
Figs 1–3

Diagnosis. Medium in size (378–427 μm in length); rostrum with deep incision reaching level of rostral setal insertion; prodorsal costula long, slightly sigmoid, diverging proximally, but converging medially and again very slightly diverging anteriorly; sensillus smooth, with relatively long stalk and slender, lanceolate head; rostral seta barbed, lamellar and interlamellar setae smooth; prodorsal tubercles *Ea* small, *Ep* large; interbothridial region with one pair of tubercles; exobothridial region with small granular tubercles; notogastral setae long, thin.

Measurements. Holotype: body length 384 μm, length of notogaster 256 μm, width of notogaster 201 μm; paratypes (n = 3) body length 378–427 (405) μm; length of notogaster 250–281 (266) μm; width of notogaster 192–213 (204) μm.

Integument. Body color yellowish brown to light brown. Surface of body and leg segments with very thin, nearly smooth cerotegument. Integument microtuberculate on tubercles, prodorsum, lateral part of prodorsum, notogaster and around leg acetabula.

Prodorsum (Figs 1A, C, D, 2A, B, 3A, B)**.** Rostrum with deep U-shaped incision reaching level of rostral setal insertion in dorsal view, but distinctly projecting anteroventrally in lateral view (Figs 1A, D, 2A). Rostral seta (*ro*) 30–36 μm long, barbed,

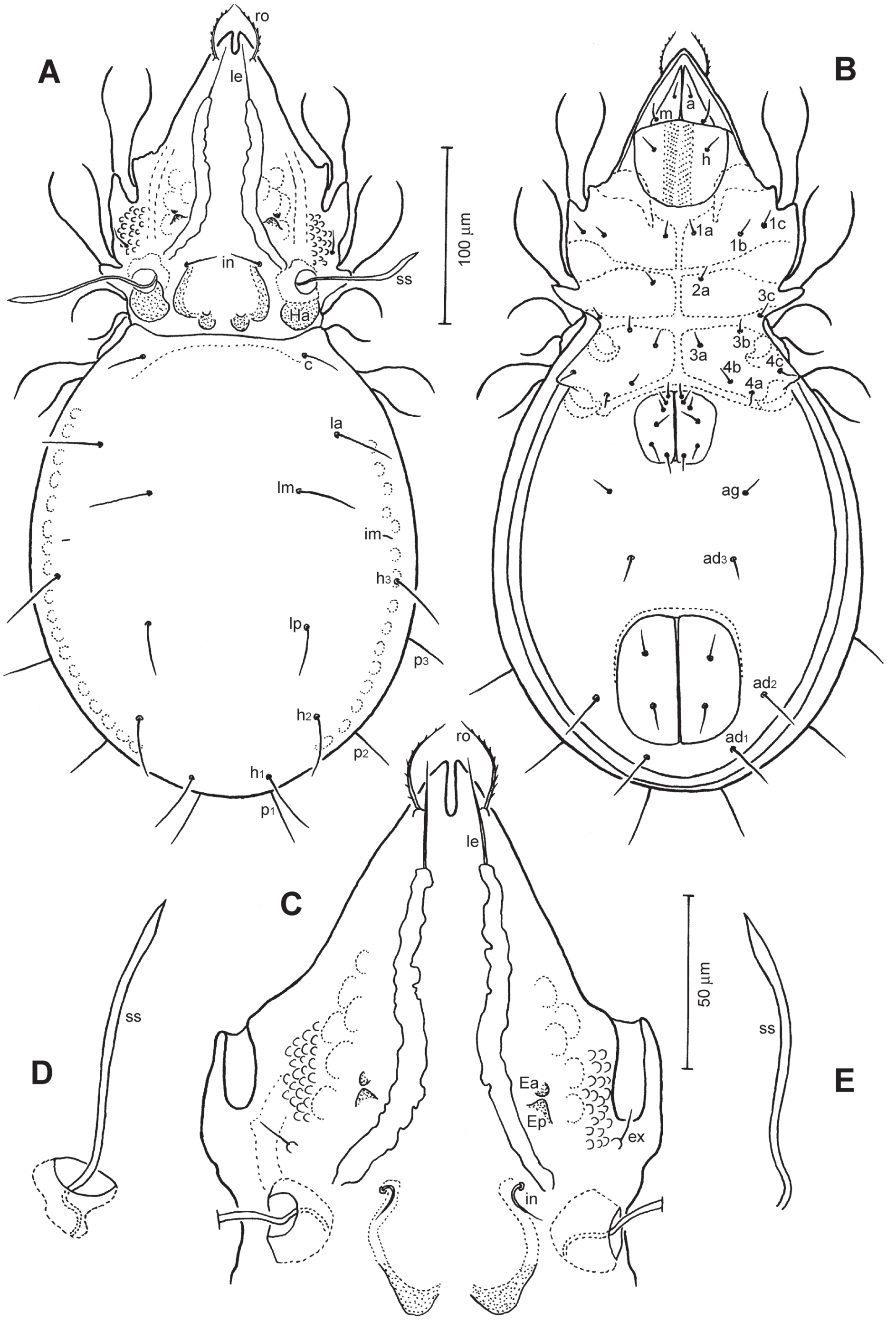

Figure 1. *Conchogneta glabrisensillata* sp. n. **A** Dorsal view of idiosoma **B** Ventral view of idiosoma **C** Prodorsum **D** Sensillus and bothridium, lateral view **E** Slight variation of sensillus, lateral view.

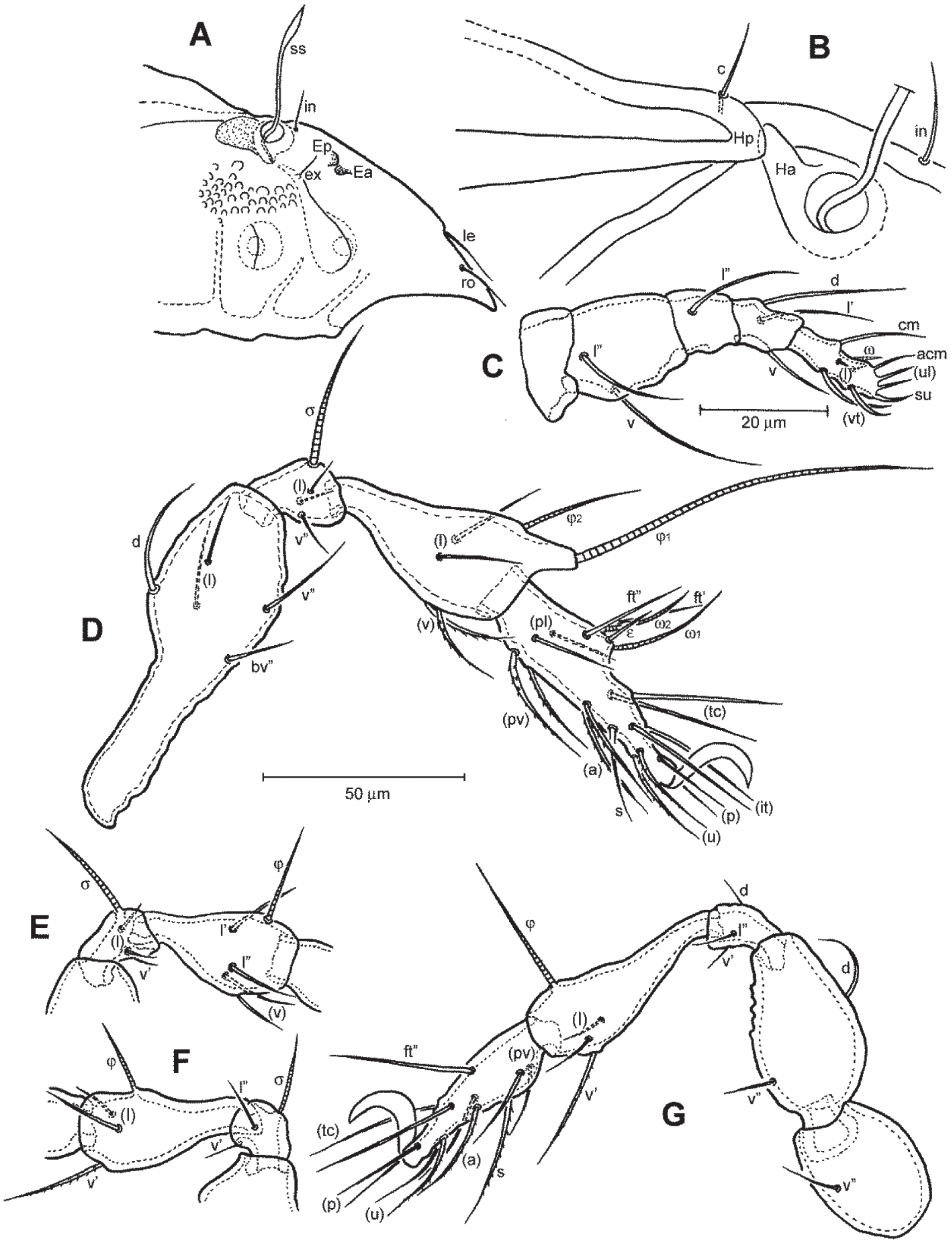

Figure 2. *Conchogneta glabrisensillata* sp. n. **A** Lateral view of prodorsum and anterior part of notogaster **B** Humeral region, showing tubercles *Ha* and *Hp* **C** Palp, right, antiaxial view **D** Leg I, right, antiaxial view **E** Genu and tibia of leg II, right, antiaxial view **F** Genu and tibia of leg III, right, antiaxial view **G** Leg IV, right, antiaxial view.

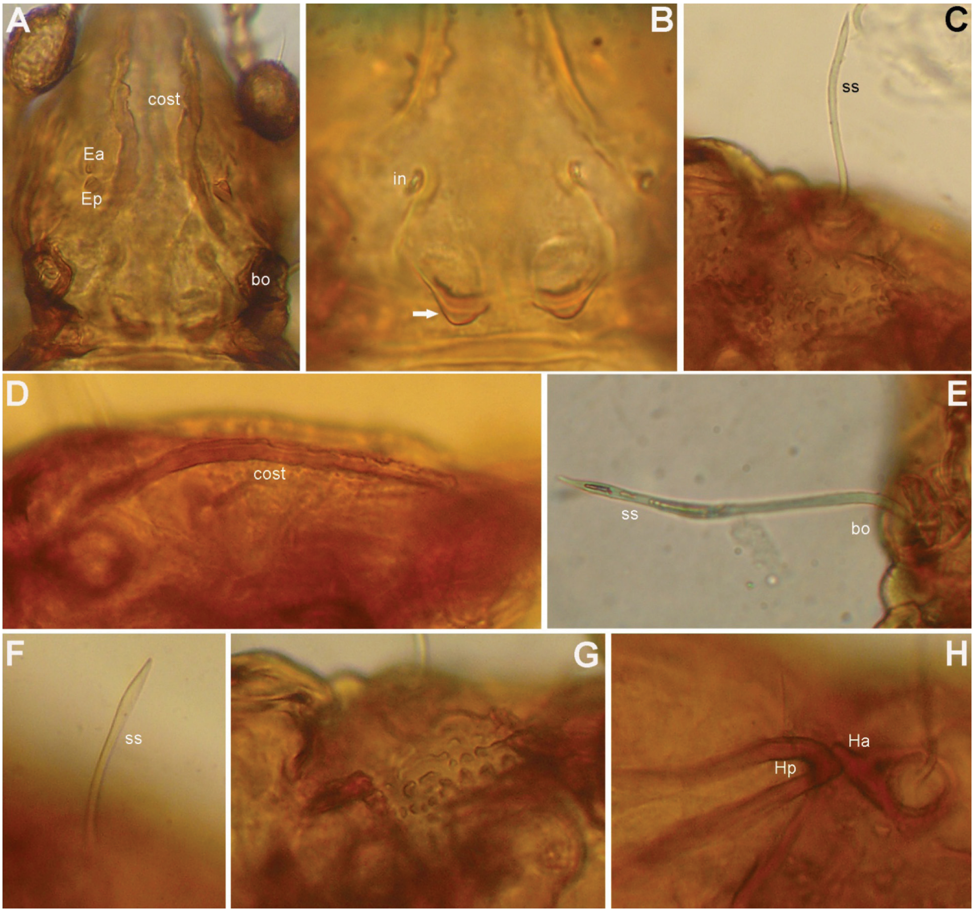

Figure 3. *Conchogneta glabrisensillata* sp. n. **A** Prodorsum, showing enantiophysis *E*, costula and bothridium **B** Central part of prodorsum, showing alveolus of interlamellar seta and interbothridial tubercle (indicated by arrow) **C** Part of lateral view of prodorsum, showing sensillus and granular tubercles on humeral region **D** Lateral view of prodorsal costula **E** Sensillus, lateral view **F** Slight variation of sensillus, lateral view **G** Granular tubercles on lateral part of prodorsum **H** Humeral region, showing tubercles *Ha* and *Hp*.

curved medially, inserted dorsally on distinct tubercle. Prodorsal costula long, slightly sigmoid, diverging proximally, but converging medially and again very slightly diverging anteriorly (Figs 1C, 3A, D). Lamellar seta (*le*) thin, smooth, 31–38 µm long, straight, inserted at distal end of costula. Interlamellar seta (*in*) 15–21 µm long, attenuate, smooth; distance between alveoli of *in-in* greater than that of *ro-ro* as viewed in dorsal aspect (Fig. 1A, D). Exobothridial seta (*ex*) inserted on distinct tubercle, 11–13 µm in length, smooth, directed anterolaterally. Sensillus (*ss*) with relatively long stalk and slender, smooth, lanceolate head; exposed portion of sensillus 70–83 µm in length (Figs 1D, E, 3C, E, F). Bothridium (*bo*) large, its opening directed posterolaterally, with large protuberance (tubercle *Ha*) posteriorly (Figs 1A, 2A, B, 3H). Prodorsal enantiophysis *E* well developed, *Ea* small, but well observable; *Ep* large, subtriangular in shape (Figs 1C, 2A, 3A). Interbothridial region with one pair of tubercle, nearly semicircular as viewed in dorsal aspect (Figs 1A, 3B).

Notogaster (Figs 1A, 2B). Oval, slightly narrowed anteriorly, about 1.3 times as long as wide. Anterior margin nearly straight, with large humeral protuberance (tubercle *Hp*; Figs 2B, 3H); posterior margin evenly rounded as viewed in dorsal aspect (Fig. 1A). Notogastral setae medium long (29–38 µm in length), thin, smooth, not reaching level of insertions of next setal row. Lyrifissure *im* well developed; other lyrifissures and opisthonotal gland opening not evident.

Gnathosoma (Figs 1B, 2C). Subcapitular mentum slightly wider than long, with minute microtubercles. Hypostomal setae *h*, *a* and *m* medium long, thin, smooth (Figs 1B). Chelicera typical for genus as shown in description of next species, slender with few slightly sclerotized blunt teeth; seta *cha* about 1.4 times as long as *chb*, both setae smooth; Trägårdh's organ small, but distinctly developed. Palp typical for genus as shown in figure 2C, palpal setation: 0-2-1-3-10, including solenidion ω of tarsus.

Epimeral region (Fig. 1B). Pedotecta I and II well developed, covered with minute granules. Epimeral region nearly smooth; setal formula 3-1-3-3, all setae medium long, smooth. Discidium well developed, projected laterally of leg acetabulum IV.

Anogenital region (Fig. 1B). Genital aperture slightly widened anteriorly, anal aperture with same width throughout. Genital, aggenital, anal and adanal setae ad_3 medium long; two other adanal setae, ad_1 and ad_2 relatively long, but all ano-genital setae thin, smooth. Adanal lyrifissure not evident.

Legs (Fig. 2D-G). Dorsal surface of claws smooth, tibia I with large dorso-distal tubercle overhanging tarsus I. Formula of leg setation (including famulus) I (1-5-3-4-18), II (1-5-3-4-15), III (2-3-2-3-15); IV (1-2-3-3-12); formula of solenidia: I (1-2-2); II (1-1-2); III (1-1-0); IV (0-1-0). Homology of leg setae and solenidia showed in Table 1.

Material examined. Holotype (female): Sevsuul valley, Eastern shore of the Lake Hövsgöl, District Khankh, Province Hövsgöl, litter of cool temperate larch forest (*Larix sibiricus* Ledebour, 1833), 51°16'N, 100°74'E, elevation 1680 m, 08 July 2007, Col. B. Bayartogtokh; three paratypes (females) same data as holotype. The holotype and one paratype are deposited in the collection of the Department of Zoology, National University of Mongolia, Ulaanbaatar, Mongolia, and two paratypes are in the collection of the Senckenberg Museum of Natural History, Goerlitz, Germany. All type specimens are preserved in alcohol.

Table 1. Leg setation of *Conchogneta glabrisensillata* sp. n.

Legs	Trochanter	Femur	Genu	Tibia	Tarsus
I	v’	d, (l), bv”, v”	(l), v”, σ	(l), (v), φ_1, φ_2	(ft), (tc), (it), (p), (u), (a), s, (pv), (pl), ε, ω_1, ω_2
II	v’	d, (l), bv”, v”	(l), v’, σ	(l), (v), φ	(ft), (tc), (it), (p), (u), (a), s, (pv), ω_1, ω_2
III	v’, l’	d, v’, l’	l”, v’, σ	(l), v’, φ	(ft), (tc), (it), (p), (u), (a), s, (pv)
IV	v”	d, v”	d, l”, v’	(l), v’, φ	ft”, (tc), (p), (u), (a), s, (pv)

Remarks. Among the eight known species of *Conchogneta*, only two of them, namely *C. traegardhi* (Forsslund, 1947) and *C. vasiliorum* Mahunka, 2006 resemble the present new species in the closely situated structure of prodorsal costulae. However, both mentioned species are different from the new species in the barbed head of sensilli as opposed to smooth sensilli in *Conchogneta glabrisensillata* sp. n. Moreover they differ in conspicuously barbed notogastral setae in contrast to smooth setae in the new species, nearly straight and thinner prodorsal costulae rather than sigmoid, but thicker costulae in the new species, and different structure of prodorsal tubercles *Ea* and *Ep*.

The other species, such as *C. dalecarlica* (Forsslund, 1947), *C. inundata* (Winkler, 1957), *C. iranica* Akrami, 2008, *C. willmanni* (Dyrdowska, 1929), *C. willmanni herzegowiensis* (Willmann, 1941) and *C. weigmanni* Mahunka, 2007 are easily distinguishable from the new species by the widely spaced prodorsal costulae, and different structure of prodorsal enantiophyses *E*.

Etymology. The specific epithet "*glabrisensillata*" refers to the smooth sensillus or bothridial seta in the new species.

Conchogneta traegardhi (Forsslund, 1947)

http://species-id.net/wiki/Conchogneta_traegardhi
Figs 4–6

Autogneta trägårdhi Forsslund 1947, p. 114, fig. 3a, b.
Autogneta traegardhi: Golosova 1975, p. 224, fig. 530.
Conchongeta traegardhi: Subías 2004, p. 109; Mahunka 2006, p. 68, Figs 12–14; Weigmann 2006, p. 318, fig. 167c-e.
Autogneta (Autogneta) traegardhi: Subías 2010, p. 195.

Diagnosis. Medium in size (353–387 µm in length); rostrum with deep incision reaching level of rostral setal insertion; prodorsal costula long, nearly straight, diverging proximally, but parallel anteriorly; sensillus with relatively long stalk and lanceolate head with few barbs at distal part; rostral seta barbed, lamellar and interlamellar setae smooth; prodorsal tubercles *Ea* and *Ep* small, same in size; interbothridial region with one pair of tubercles; exobothridial region with small granular tubercles; notogastral setae long, thin.

Measurements. Body length 353–387 (368) µm; length of notogaster 225–251 (236) µm; width of notogaster 186–205 (198) µm.

Integument. Body color yellowish brown to light brown. Surface of body and leg segments with very thin, nearly smooth cerotegument. Integument microtuberculate on tubercles, prodorsum, lateral part of prodorsum, notogaster and around leg acetabula.

Prodorsum (Figs 4A, C, D, 5A, 6). Rostrum with deep U-shaped incision reaching level of rostral setal insertion in dorsal view, but distinctly projecting anteroventrally in lateral view (Figs 4A, C, 5A). Rostral seta 28–33 µm long, barbed, curved medially,

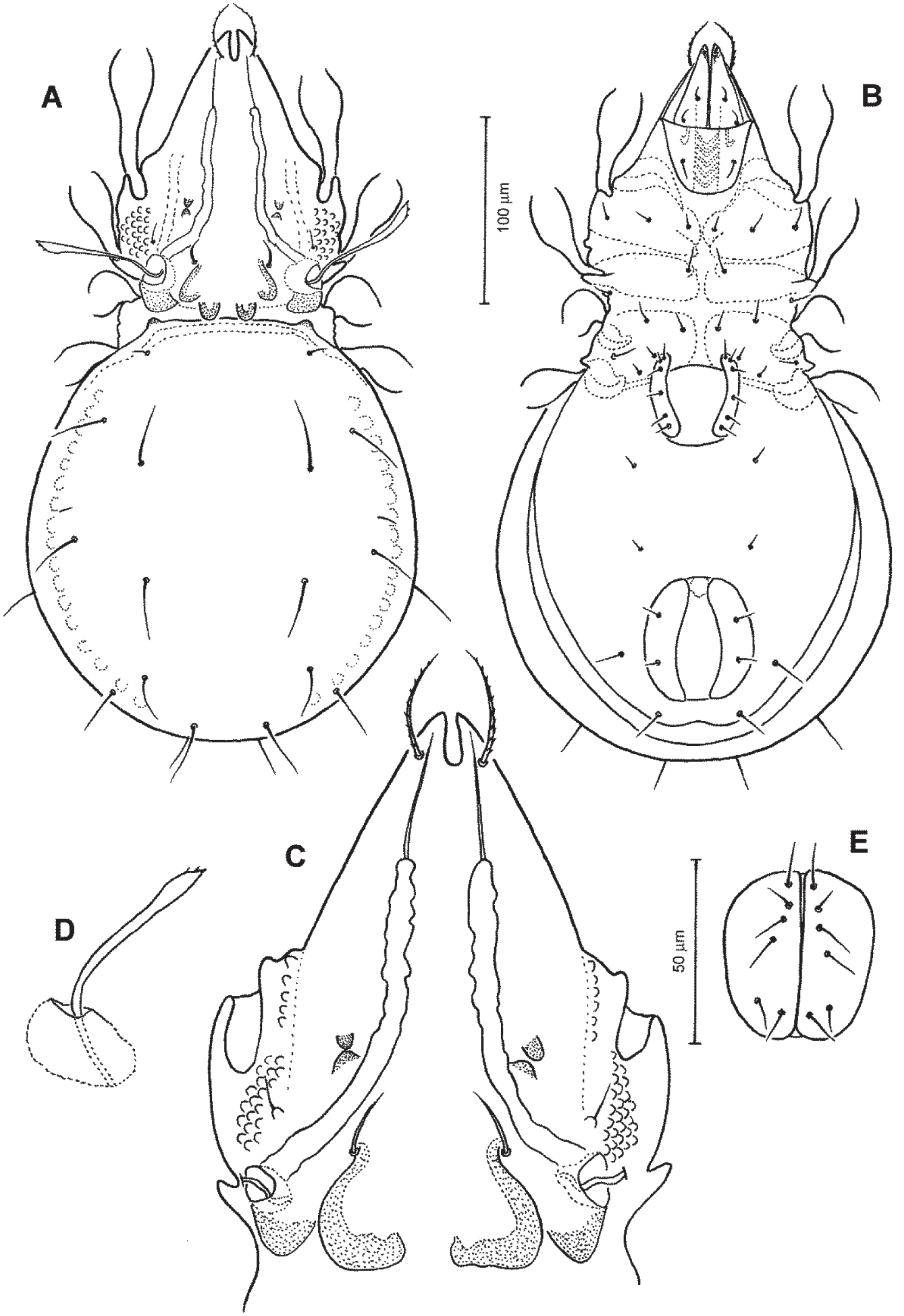

Figure 4. *Conchogneta traegardhi* (Forsslund, 1947). **A** Dorsal view of idiosoma **B** Ventral view of idiosoma **C** Prodorsum **D** Sensillus and bothridium, lateral view **E** Genital plate.

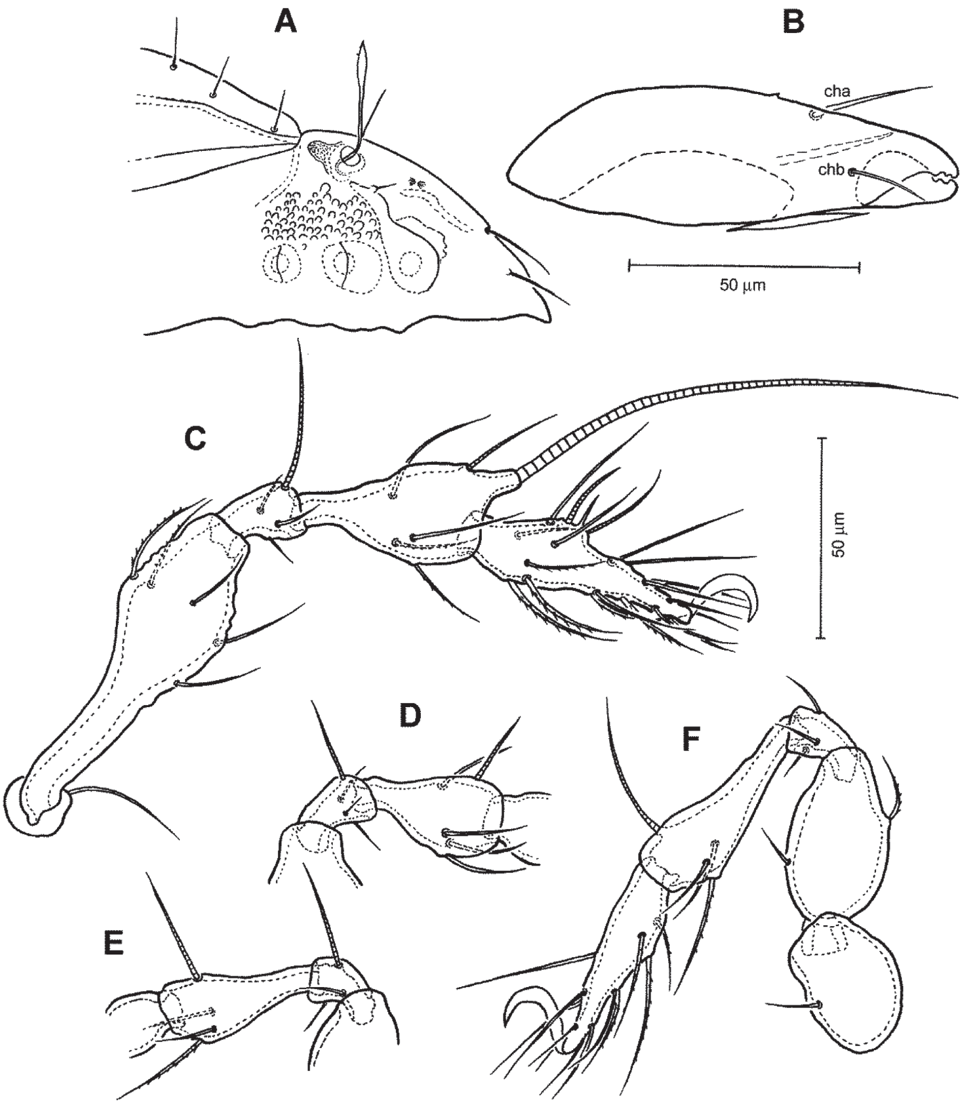

Figure 5. *Conchogneta traegardhi* (Forsslund, 1947). **A** Lateral view of prodorsum and anterior part of notogaster **B** Chelicera, right, antiaxial view **C** Leg I, right, antiaxial view **D** Genu and tibia of leg II, right, antiaxial view **E** Genu and tibia of leg III, right, antiaxial view **F** Leg IV, right, antiaxial view.

inserted dorsally on distinct tubercle. Prodorsal costula long, nearly straight, diverging proximally, but parallel anteriorly (Figs 4A, C, 6D). Lamellar seta thin, smooth, 30–34 µm long, straight, inserted at distal end of costula. Interlamellar seta 24–29 µm long, attenuate, smooth; distance between alveoli of *in-in* greater than that of *ro-ro* as viewed in dorsal aspect. Exobothridial seta inserted on distinct tubercle, 10–13 µm in length, smooth, directed anterolaterally (Fig. 4C). Sensillus with relatively long stalk

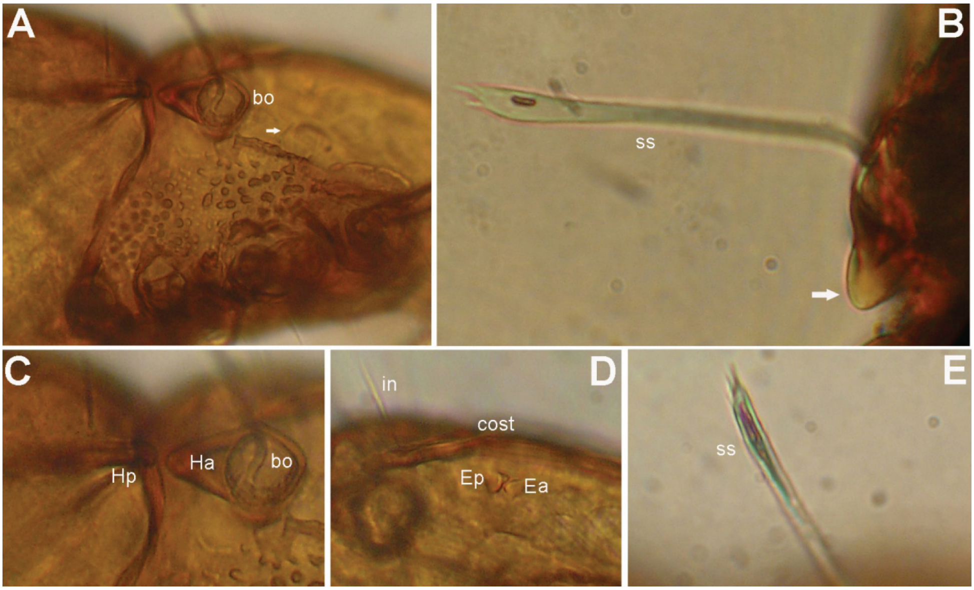

Figure 6. *Conchogneta traegardhi* (Forsslund, 1947). **A** Lateral view of prodorsum, arrow indicates exobothridial seta **B** Sensillus and bothridium, lateral view, arrow indicates postbothridial tubercle *Ha* **C** Humeral region, showing tubercles *Ha* and *Hp* and bothridium **D** Lateral view of part of prodorsum showing prodorsal costula, enantiphysis *E* and interlamellar seta **E** Slight variation of sensillus, lateral view.

and lanceolate head with two or three barbs; exposed portion of sensillus 70–80 µm in length (Figs 4D, 6B, E). Bothridium large, its opening directed posterolaterally, with large protuberance (tubercle *Ha*) posteriorly (Figs 4C, 5A, 6B, C). Prodorsal enantiophysis *E* well developed, tubercle *Ea* and *Ep* small, same in size, subtriangular in shape (Figs 4A, C, 5A). Interbothridial region with one pair of tubercle, nearly semicircular as viewed in dorsal aspect (Figs 4A, C).

Notogaster (Figs 4A, 5A, 6A, C). Oval, slightly narrowed anteriorly, about 1.2 times as long as wide. Anterior margin nearly straight, with large humeral protuberance (tubercle *Hp*; Fig. 6C); posterior margin evenly rounded as viewed in dorsal aspect. Notogastral setae medium long (29–35 µm in length), thin, smooth, not reaching level of insertions of next setal row (Fig. 4A). Lyrifissure *im* well developed; other lyrifissures and opisthonotal gland opening not evident.

Gnathosoma (Figs 4B, 5B). Subcapitular mentum slightly wider than long, with minute microtubercles. Hypostomal setae *h*, *a* and *m* medium long, thin, smooth (Figs 4B). Chelicera slender with few slightly sclerotized blunt teeth; seta *cha* about 1.4 times as long as *chb*, both setae smooth; Trägårdh's organ small, but distinctly developed (Fig. 5B). Palp typical for genus as shown in previous species, palpal setation: 0-2-1-3-10 including solenidion ω of tarsus.

Epimeral region (Fig. 4B). Pedotecta I and II well developed, covered with minute granules. Epimeral region nearly smooth; setal formula 3-1-3-3, all setae medium long, smooth. Discidium well developed, projected laterally of leg acetabulum IV.

Anogenital region (Fig. 4B). Genital aperture slightly widened anteriorly, anal aperture with same width throughout. Genital, aggenital, anal and adanal setae ad_3 medium long; two other adanal setae ad_1 and ad_2 relatively long, but all ano-genital setae thin, smooth. Adanal lyrifissure not evident.

Legs (Fig. 5C–F). Dorsal surface of claws smooth, tibia I with large dorso-distal tubercle overhanging tarsus I. Formula of leg setation (including famulus) I (1-5-3-4-18), II (1-5-3-4-15), III (2-3-2-3-15); IV (1-2-3-3-12); formula of solenidia: I (1-2-2); II (1-1-2); III (1-1-0); IV (0-1-0).

Material examined. Thirty-six specimens: Khuitnii-Am area, Mts Mongol Altai, close to Lake Dayan, District Sagsai, Province Bayan-Ulgii, litter of cool temperate larch forest (*Larix sibiricus* Ledebour, 1833), N48°14', E88°55', elevation 2356 m a.s.l., 18 July 2010; four specimens: same data as above, but from fruticose lichens growing on larch tree barks, 01 August 2010; six specimens: same data as above, but from soil-litter of forest edge, 17 July 2010; three specimens: same data as above, but from soil of steppe, 27 July 2010, Col. B. Bayartogtokh.

Remarks. The characters of specimens studied here from Mongolia are match well with the specimens studied by Forsslund (1947), Golosova (1975), Mahunka (2006) and Weigmann (2006). For the sake of completeness, I provide supplementary descriptions with detailed illustrations.

Discussion

The members of the oribatid mite genus *Conchogneta* are limited to the Northern Hemisphere and are mainly known from moist, warm soils and litter habitats in temperate regions. However, the diversity of this genus is not high, as most of the species were recorded in Europe, except three species, which have expanded distributions in Asia (three species) and North America (one species). Most species of *Conchogneta* are inhabitants of various type forest litters (beech, birch, fir, spruce, pine, larch), bryophytes (*Hypnum* sp., *Sphagnum* sp.) growing on the forest floor, in cave or as epyphytes on hazel trees, as well as soils in river valleys, moor, oligotrophic bogs, floodland assemblages (Weigmann and Kratz 1982, Beck and Woas 1991; Huhta and Niemi 2003, Sidorchuk 2009; Toluk and Ayyildiz 2009).

The genus is represented in Mongolia with only two species studied here, and one of them, *C. traegardhi* is the most widely distributed species of *Conchoneta*, which is known from Palaearctic and Nearctic regions. It should be noted here that Subías (2010) removed this species from *Conchogneta* and included it in the genus *Autogneta* Hull, 1916, without any commentary. However, the structure of the sensilli speaks against the inclusion of *C. traegardhi* in *Autogneta*, as the other species of *Autogneta* have clavate or capitate sensilli in contrast to lanceolate or fusiform sensilli in *C. traegardhi*, which is typical of *Conchogneta* (Weigmann 2006). Moreover, according to Grandjean (1963), the genera *Autogneta* and *Conchogneta* generally differ in their ontogeny, with the juvenile stages of latter exhibiting spatulate-pateriform setae on both the prodorsum and notogaster.

C. traegardhi is known to be a sylvicolous species, widely distributed in Eurasia and North America, but it is nowhere common (Mahunka 2006). However, it is one of the dominating species in the Mongol Altai Mountains, where I found it abundantly in the litter of the interior of larch forests. It occurs with up to 28 individuals per 125 cm^3 of soil-litter samples collected in the forest interior, but was rarely found in the forest edge or steppe soils, where less than 4 individuals per sample were found. The livestock primarily grazes in the steppe, but also forest margins and less intensively the interior of forests are utilized for pasture. Additionally, were also collected a few individuals of *C. traegardhi* from the fruticose lichens (*Xanthoria candelaria*, *Rhizoplaca chrysolenca*, *Parmeliopsis ambigua*) growing at the trunk base of larch trees. Most specimens of the present species had food in their gut and food boluses primarily contained fungal hyphae. Heggen (2010) revealed *C. traegardhi* as an inhabitant of the lower zones of alpine regions in Fennoscandia, but did not find it in the higher alpine zones. Therefore, Heggen (2010) concluded that the distribution of *C. traegardhi* might be limited by altitude. However, this species is abundantly occurred in the high alpine zone of the Mongoli Altai Mountains at elevation of more than 2300 m above sea level. Therefore, I suggest that the upper limit distribution of this species depends more on the occurrence of forests than on elevation (and, with it, temperature) itself.

The second species found in Mongolia, *Conchogneta glabrisensillata* sp. n. is quite rare, and was only found in a few samples of single valley out of six studied valleys in the eastern tributaries of Lake Hövsgöl, where many soil, litter and lichen samples were investigated. The valley is a broad, flat valley with steppe vegetation covering the valley bottom and south-facing slopes of mountains on the north side of the valley. The extensive larch forests cover the north facing slopes of the mountains. The valley floor consists of sandy soils and the river sediment is also very sandy. However, the forest floor has a fairly thick litter horizon with black humus rich soil mixed with mosses and lichens. There is relatively heavy grazing with indications of excessive grazing on the south facing slopes, but less grazing pressure in the forest. The new species was collected from litter of a larch forest, and the area is very cold, but one of the moistest regions within Mongolia. While currently known only from forest litter at the type locality, *C. glabrisensillata* sp. n. probably has a restricted geographic distribution and ecological niche in cold areas.

In the regular update of the checklist of world oribatid mites, Subías (2006) treated the type species of *Conchogneta*, *C. dalecarlica* (Forsslund, 1947), as a junior synonym of *C. willmanni* (Dyrdowska, 1929). Indeed these two species are similar to each other, especially in the widely spaced prodorsal costulae. However, not only these two species, but also several other members of *Conchogneta*, including *C. inundata*, *C. weigmanni* and *C. willmanni herzegowinensis*, share the costulae laterally placed on prodorsum. The other species have closely placed costulae, which are situated along the center of prodorsum. Thus, all species of *Conchogneta* can be classified into two groups in respect to their structure of prodorsal costulae.

When he synonymized *C. dalecarlica* with *C. willmanni*, Subías (2006) did not provide any commentary or justification. In contast to Subías (2006), *C. dalecarlica* and *C. willmanni* are treated here as different species, because they differ in the structure of the sensilli and the prodorsal costulae (Mahunka 2006, Akrami 2008). This view agrees with that of Woas (1986), Weigmann (2006), Toluk and Ayyildiz (2009).

The following key can be used to identify adults of all known species of *Conchogneta*.

World-wide key to the adults of *Conchogneta*

1 Prodorsal costula widely spaced from each other, placed laterally on prodorsum, strongly converging anteriorly **2**

– Prodorsal costula closely placed to each other, situated along center of prodorsum, nearly parallel or slightly converging anteriorly **7**

2 Sensillis setiform or very slightly dilated distally **3**

– Sensillus lanceolate or pectinate **5**

3 Notogastral setae medium long, not reaching alveoli of next setal row; interlamellar seta short **4**

– Notogastral setae long, reaching alveoli of next setal row; interlamellar seta long .... ***C. wilmanni herzegowinensis*** **Willmann, 1941**

4 Sensillus with long ciliae; costula very widely spaced from each other .... ***C. iranica*** **Akrami, 2008**

– Sensillus smooth; costula relatively close to each other .... ***C. inundata*** **(Winkler, 1957)**

5 Prodorsum with one pair of basal tubercles; costula thin, without lateral oval field **6**

– Prodorsum with two pairs of basal tubercles; costula very thick, with lateral oval field .... ***C. weigmanni*** **Mahunka, 2007**

6 Sensillus very long, its head bifurcate .... ***C. willmanni*** **(Dyrdowska, 1929)**

– Sensillus relatively short, its head pectinate or well pilose .... ***C. dalecarlica*** **(Forsslund, 1947)**

7 Distal part of costula not dilated; sensillus lanceolate, distally covered with few short barbs or smooth; interlamellar seta smooth; body length smaller than 430 µm **8**

– Distal part of costula dilated; sensillus baciliform, distally covered with many short barbs; interlamellar seta barbed; body length greater than 500 µm .... ***C. vasiliorum*** **Mahunka, 2006**

8 Prodorsal tubercles *Ea* much smaller than *Ep*; sensillus smooth; anterior part of costula not straight, but slightly rounding .... ***C. glabrisensillata*** **sp. n.**

– Prodorsal tubercles *Ea* and *Ep* small, same in size; sensillus with few, but distinct barbs; anterior part of costula nearly straight .... ***C. traegardhi*** **(Forsslund, 1947)**

Acknowledgments

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RESEARCH ARTICLE

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Description of two new species of *Clivina* Latreille (Coleoptera, Carabidae, Clivinini) from southeastern United States

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Abstract

Two new species of the genus *Clivina* Latreille are described. One, *C. choatei* Bousquet & Skelley, belongs to the nominotypical subgenus and is known from six specimens collected in northern Florida. The species is structurally similar to *C. myops* Bousquet, known only from the holotype found in North Carolina, but differs among others by its smaller size and wider elytral striae. The second species, *C. alabama* Bousquet, belongs to the subgenus *Antroforceps* Barr and is known from two specimens collected in north-central Alabama. The species is structurally most similar to *C. sasajii* Ball, known only from Latimer County in Oklahoma, but differs among others in the absence of eyes and in having the pronotum and elytra proportionally wider.

Keywords

Florida, Alabama, taxonomy, *Clivina*

Introduction

The genus *Clivina* Latreille is a moderately diverse taxon represented in all continents, except Antarctica, by about 375 species. In the Nearctic Region, the genus contains 17 species of which three are adventive on this continent. These species are arrayed in five subgenera: *Paraclivina* Kult, *Clivina s.str.*, *Semiclivina* Kult, *Antroforceps* Barr, and *Leucocara* Bousquet (= *Reichardtula* Whitehead *sensu auctorum* in part). Ball (2001) provided an excellent description of the adult structural characters of the genus and a key to the subgenera represented in North America.

The purpose of this paper is to describe two new species belonging to the nominotypical subgenus of *Clivina* from northern Florida and to the subgenus *Antroforceps* from north-central Alabama.

Methods

The following measurements, made with an ocular micrometer in a Leica MZ16, were made on all specimens of the type series and from the holotype of *C. myops* and two paratypes of *C. sasajii*: head length (HL) – linear distance from apical edge of clypeus to posterior edge of left temple; length of pronotum (PL) – linear distance from anterior to posterior edge, measured along the midline; width of pronotum (PW) – greatest linear transverse distance; length of elytra (EL) – linear distance from parascutellar seta to apex, measured along the midline; width of elytra (EW) – greatest linear transverse distance across both elytra. The standardized body length (SBL) is the sum of the lengths of head, pronotum, and elytra, as specified above. The apparent body length (ABL) is the length measured from the apex of the mandibles to the apex of the abdomen.

Taxonomy

Clivina (*Clivina*) *choatei* Bousquet & Skelley, sp. n.

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http://species-id.net/wiki/Clivina_choatei
Fig. 1

Type material. Holotype, male, labeled: “FLORIDA: Levy Co. 4.0mi SW Archer 20-IX-30-XII-2001 P. Skelley, panel BIT / Holotype Clivina choatei Bousquet & Skelley.” The holotype is in the Florida State Collection of Arthropods, Gainesville, Florida (FSCA). Paratypes (five specimens): one specimen labeled “FLORIDA: Levy Co. 4.0mi SW Archer on Rt.24; 1-16-V-2001 P. Skelley, panel BIT”; 2 specimens labeled “FLORIDA: Levy Co. 4.0mi SW Archer 25-I-1992; Heyer, Skillman, Skelley Geomys chambers”; 2 females labeled “Gilchrist Co. N. Bell, 1mi.S.Rt.340 on Rt.129/40; 4-XII-1997 to 20-III-1998; P. Skelley *Geomys* burrow pitfall.” All paratypes are also labeled “Paratype Clivina choatei Bousquet & Skelley” and are deposited in FSCA (4 specimens) and in the Canadian National Collection of Insects, Ottawa, Ontario (1 specimen).

Etymology. The specific epithet is a Latinized singular noun in apposition, genitive case, based on the surname of Paul “Skip” Choate (Department of Entomology

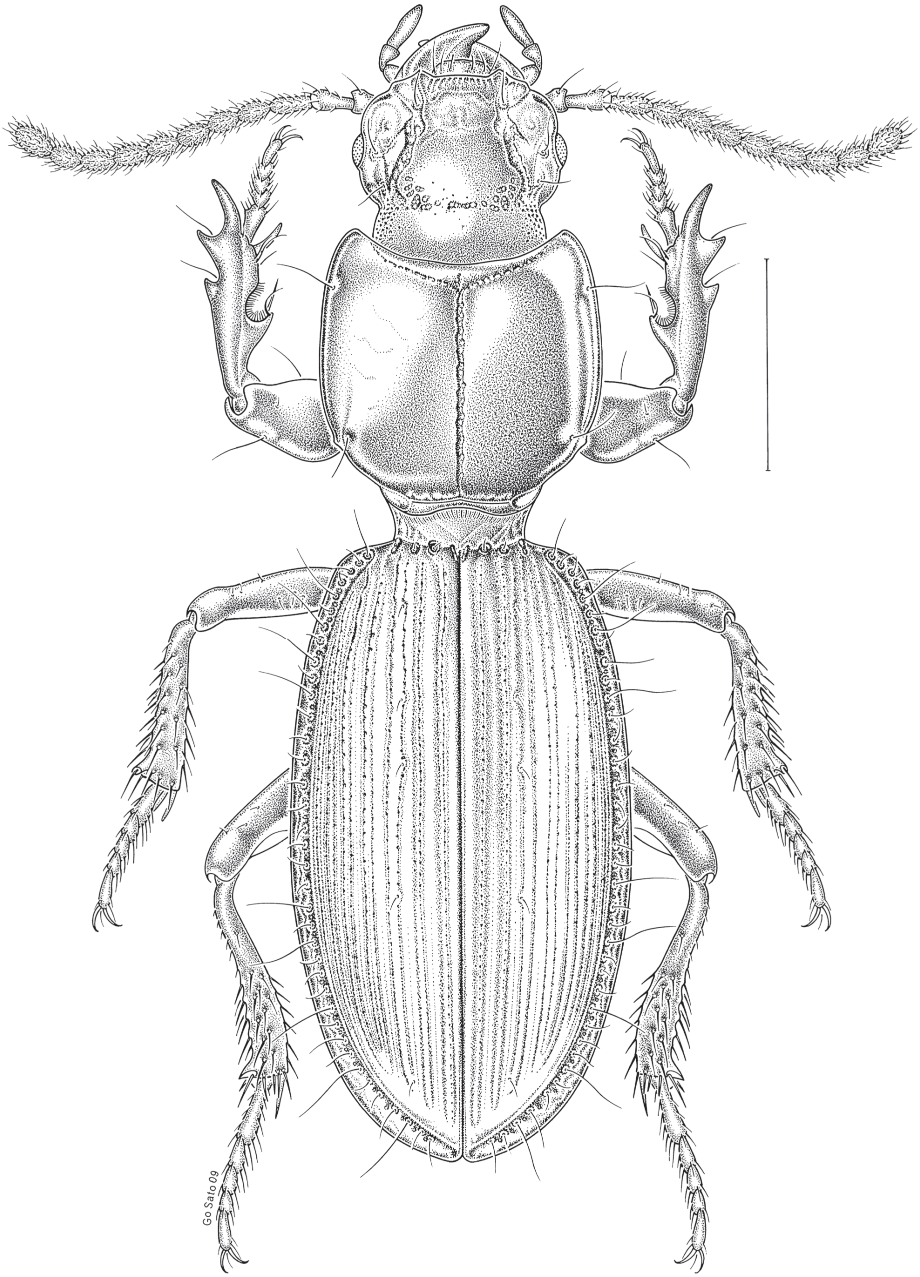

Figure 1. *Clivina choatei*, habitus (dorsal view); scale bar = 1 mm.

& Nematology, University of Florida, Gainesville), who first collected this species and showed the specimens to PES. Unfortunately, his original specimens were lost.

Description. *Color*. Body rufous with median and posterior legs slightly paler. *Microsculpture*. Clypeus and frons without meshes. Pronotum without meshes except at extreme base. Elytra without meshes except along intervals 8. Visible abdominal sternites 2–5 without meshes between ambulatory setae; visible sternite 6 with band of faint isodiametric meshes between ambulatory setae; visible sternites 1–2 with coarse, more or less isodiametric meshes laterally. *Head*. Labrum with six or seven dorsal setae. Clypeus with anterior margin almost straight, with very small lateral dentiform projections. Frontoclypeal suture indistinct. Frons without median fovea. Anterior supraorbital seta near level of posterior edge of eye. Eye small, flat. Antennomere 2 subequal in length to antennomere 3; antennomere 2 with several setae, most located in apical half; antennomeres 6–10 subquadrate. Mentum tooth proportionally large, not acuminate, apex more or less rounded, not quite reaching apex of lobe. *Thorax*. Pronotum with disc convex, with few faint wrinkles, without conspicuous punctures; lateral edges subparallel between level of setigerous punctures, with single denticle in posterior third; anterior angles protruding; median longitudinal impression shallow but relatively wide; anterotransverse impression distinct except near median impression, deep. Proepisternum with punctures on anterior half. Metepisternum with punctures. Metasternum smooth except for a few punctures laterally. *Elytra*. Striae wide, as wide or almost so as corresponding intervals, sides of striae wavy. Intervals flat; intervals 3 and 4 with conspicuous protuberance each at extreme base; interval 3 with five setigerous punctures; intervals 6 and 7 not carinate near base. Lateral edge along humeral region smooth, without indentations. *Abdomen*. Visible abdominal sternite 2 without coxal lines; visible sternites with coarse, shallow but dense punctures laterally. *Legs*. Protarsomere 1 with minute process on lateral side. *Male genitalia*. Median lobe with apex spatulate; endophallus without spines.

SBL = 4.45–5.05 mm.

Habitat. Although some specimens were collected in pitfall traps set in pocket gopher (*Geomys*) burrows, others were not. Choate's missing specimens from western Alachua County were collected while excavating pocket gopher burrows, but may not have been in the burrows. The holotype and paratypes collected in "panel BIT" were far away from any rodent burrows. "BIT" stands for "burrow intercept trap" and was one of many passive underground trap designs the second author experimented with to collect subterranean insects (see Bousquet and Skelley 2010). Underground pitfalls sampling pocket gopher burrows sometimes act like a BIT and can collect other subterranean insects. Specimens of *C. choatei* and *C. sasajii* were collected in association with pocket gophers. This loose association is felt to be opportunistic for the beetle and a sampling bias by collectors looking for other underground insects.

All specimens of *C. choatei* were collected in deep sandy soils of Levy and Gilchrist counties which are part of the Northern Brooksville Ridge. This is one of many isolated sand systems in the southeastern US, which are known for having many endemic plants and animals. A brief discussion of this area is given in Bousquet and Skelley (2010).

Geographical distribution. This species is currently known only from Levy and Gilchrist Counties on the Florida Panhandle.

Remarks. This species is structurally very similar to *Clivina myops*, which is known only from the holotype collected at Raleigh in North Carolina. The holotype of *C.*

myops differs from specimens of *C. choatei* by its larger size (SBL = 6.40 mm), in having the median longitudinal impression of the pronotum deeper, the elytral striae proportionally narrower, their width being clearly less than that of the corresponding intervals, and the visible abdominal sternites 1 and 2 less punctate, with only a few, relatively small punctures. The ratios of various body proportions (Table 1) suggest that the pronotum is proportionally wider in *C. choatei* than in *C. myops*. The shape of the median lobe of the aedeagus did not differ significantly between the two species.

In Bousquet's (1997) key to the Nearctic species of the subgenus *Clivina*, *C. choatei* will key out to couplet 6. The following modification should be made to incorporate the new species:

6 Antennomere 2 at most with one seta on apical half. Proepisternum smooth or at most with a few punctures .... ***C. pallida* Say**

– Antennomere 2 with several setae on apical half. Proepisternum punctate on anterior half **6A**

6A Elytral striae proportionally narrower, width smaller than that of corresponding intervals. Body larger, ABL = 7.0 mm .... ***C. myops* Bousquet**

– Elytral striae proportionally wider, width subequal to that of corresponding intervals. Body smaller, ABL = 4.8–5.5 mm........................ ***C. choatei* sp. n.**

Table 1. Ratios for *Clivina* studied.

species	N	HW/PW	PW/EW	PL/PW	PL/EL	EL/EW
C. choatei	6	0.72–0.80	0.82–0.90	0.92–0.95	0.39–0.41	1.90–2.05
C. myops	1	0.79	0.77	0.98	0.39	1.92
C. alabama	2	0.70–0.78	0.86	0.96–0.98	0.45–0.47	1.79–1.84
C. sasajii	2	0.73–0.75	0.87–0.92	1.11–1.17	0.47–0.48	2.13–2.17

***Clivina* (*Antroforceps*) *alabama* Bousquet, sp. n.**

urn:lsid:zoobank.org:act:4937B489-B81C-496F-A78F-5925D330D7F6
http://species-id.net/wiki/Clivina_alabama
Fig. 2

Type material. Holotype labeled: "AL: 0.5 mi S Highland Lake Blount Co. Oct. 22, 2009 T.N. King / Blind Carabid HL - 10.22.09 Rock 1000' [handwritten] / Holotype Clivina alabama Bousquet CNC No. 24034." The holotype is in the Canadian National Collection of Insects, Ottawa, Ontario. Paratype (1 specimen) labeled: "AL: 0.5 mi S Highland Lake Blount Co. April 2009 T.N. King / Paratype Clivina alabama Bousquet." The specimen is deposited in R. Michael Brattain Collection (Lafayette, Indiana).

Etymology. The specific epithet is a Latinized singular noun in apposition, nominative case, based on the name of the Alabama, a native American tribe who lived in southeastern United States.

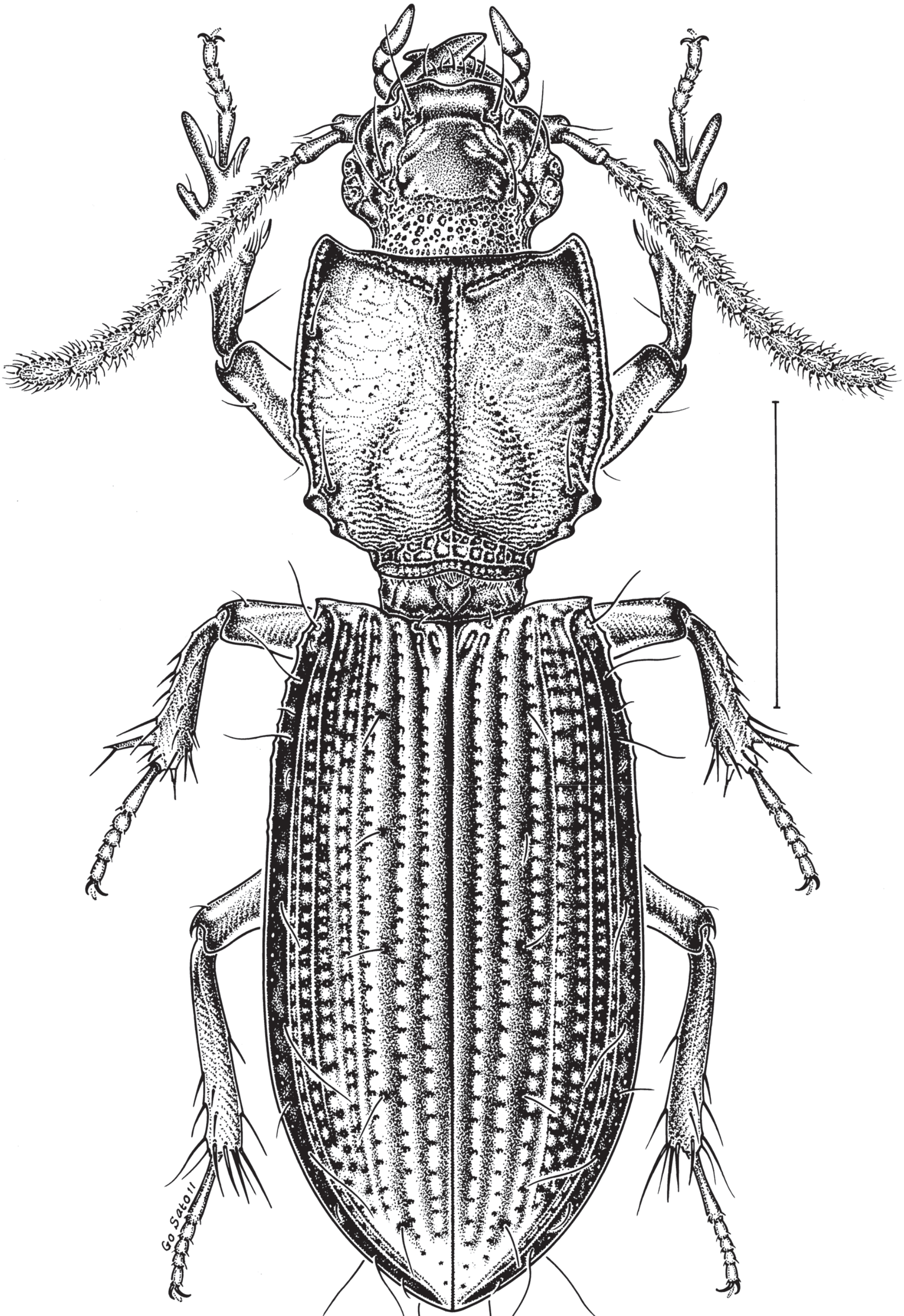

Figure 2. *Clivina alabama*, habitus (dorsal view); scale bar = 1 mm.

Description. With the character states of the subgenus *Antroforceps* as described by Ball (2001: 138–144) and the followings: *Color*. Body uniformly rufous, with antennae, palpi and most of legs paler. *Microsculpture*. Frons and clypeus without microsculpture. Pronotum without microsculpture except for small area near the posteriolateral dentiform projections. Elytra without microsculpture. Proepisternum with coarse, isodiametric meshes, sculpticells convex. Visible abdominal sternites 1–6 with coarse isodiametric meshes except medially on sternites 2–4, sculpticells convex. *Head*. Clypeus with anterior margin slightly concave medially, without dentiform projections laterally. Frontoclypeal suture very shallow. Frons without median fovea. Eye absent. Antennomeres 6–10 submoniliform. Mentum tooth proportionally small, apex more or less rounded, not reaching apex of lobe. *Thorax*. Pronotum slightly transverse (see Table 1); lateral edges shallowly crenulate; anterior angles acutely protruding; median longitudinal impression narrow along posterior half, widened along anterior half; posteriolateral dentiform projections blunt, basal projection as large as apical projection. *Elytra*. Lateral edges minutely crenulate on basal fourth; interval 4 convex in basal fourth; interval 5 rather flat; interval 6 and 7 carinate through most of length; interval 3 with five setigerous punctures.

SBL = 3.63–3.69 mm.

Habitat. The two specimens were collected under rocks on a hillside in a mixed Pine/hardwood area above a stream following heavy rains (R. Michael Brattain, personal communication 2011).

Geographical distribution. This species is currently known only from Blount County in north-central Alabama.

Remarks. This species is structurally most similar to *Clivina sasajii*, which is known from nine specimens collected in Latimer County, eastern Oklahoma. The two paratypes of *C. sasajii* studied differ from those of *C. alabama* in having small eyes, the pronotum clearly elongate (see Table 1) and the elytra proportionally narrower (see Table 1), the anterior angles of the pronotum less projected, the posteriolateral dentiform projections more acute, with the basal projection smaller than the apical one, the microsculpture near the posteriolateral dentiform projections more expanded, the lateral edges of the elytra indistinctly crenulate on basal fourth, the interval 4 not convex, and the interval 6 carinate only on anterior fourth. The genitalia of the two specimens of *C. alabama* have not been extracted to preserve their integrity.

In Ball's (2001: 144–145) key to the species of subgenus *Antroforceps*, *C. alabama* will key out to couplet 2. The following modification should be made to incorporate the new species:

2 Elytral interval 3 with eight discal setae. Lateral margins of pronotum pronouncedly crenulate. Pronotum and elytra with paramedian dorsal projections .... ***C. bolivari* Barr**

– Elytral interval 3 with five discal setae. Lateral margins of pronotum shallowly crenulate. Pronotum and elytra without paramedian dorsal projections **3**

3 Eyes absent. Pronotum slightly transverse (PL/PW = 0.96–0.98); elytra proportionally wider (EL/EW = 1.79–1.84)........................... ***C. alabama*** **sp. n.**

– Eyes present, small. Pronotum elongate (PL/PW = 1.11-1.17); elytra proportionally narrower (EL/EW = 2.13–2.17)..................................***C. sasajii*** **Ball**

Acknowledgements

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RESEARCH ARTICLE

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A remarkable new species of *Euragallia* from Peru (Insecta, Hemiptera, Cicadellidae, Agalliini), including the description of a peculiar structure of the male genitalia

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Abstract

A new species of *Euragallia* Oman, 1938 from Peru (Pasco Department) is described and illustrated. *Euragallia batmani* **sp. n.** can be distinguished from the other species of the genus by the very posteriorly pronounced male pygofer, with an apical hook-like projection, and by the well-developed dorsal area of the aedeagal base, resembling the open wings of a bat. With the addition of *E. batmani* sp. n., the number of *Euragallia* species is increased to 21. Only one additional species of the genus is recorded from Peru (*E. prion* Kramer, 1976). A comparison between the new species and *E. prion* is provided. A conspicuous structure, which connects the subgenital plates to the styles, is described in detail and named.

Keywords

Leafhopper, Megophthalminae, Membracoidea, morphology, South America, taxonomy

Introduction

The subfamily Megophthalminae comprises approximately 53 genera and 650 species distributed in four tribes. In the Neotropical Region, about 22 genera and 280 species are known. The tribes are distributed as follows: Adelungiini has a Palearctic distribution; Megophthalmini, African and Holarctic; Evansiolini, restricted to Chile (Juan Fernandez Islands); and Agalliini (traditionally treated as a separate subfamily), cosmopolitan. The latter tribe, which is the largest within the subfamily and includes the genus herein treated, was historically studied mostly by Oman (1933, 1934, 1938a), Linnavuori (1954), and Kramer (1964, 1976). Species in this group occur primarily in dense, low-growing herbaceous or shrubby vegetation (Gonçalves and Nielson 2011).

Euragallia Oman, 1938 (type-species *Agallia furculata* Osborn, 1923) appears to be limited to the tropical and subtropical regions of the Western Hemisphere, being recorded from Mexico to Brazil (Kramer 1964). Oman (1938a) carried out a revision of the genus. Other meaningful contributions were made by Kramer (1964, 1976), Linnavuori (1968), Cwikla and DeLong (1985), Dutra and Coelho (1992), Nielson and Godoy (1995), and Gonçalves and Zanol (2010). As a result of these studies, *Euragallia* contains currently 20 described species (Gonçalves and Zanol 2010).

The most relevant diagnostic characters of *Euragallia* are as follows: (1) robust species, their coloration being brown or fuscous, never with bright spots; (2) crown shorter on median region than next to eyes; (3) eyes usually bulbous; (4) subgenital plates always fused to each other at least basally, frequently small, exposing apexes of styles; (5) styles never forked; (6) abdominal segment X usually well-developed; (7) seventh sternite of female reduced, sometimes exposing base of ovipositor (Linnavuori 1954, Kramer 1964, Gonçalves and Zanol 2010). In a recently published paper, Gonçalves and Zanol (2010) recognized three groups into which species of *Euragallia* can be segregated: *declivata*, *magnicauda*, and *major*.

In this paper, a new species of *Euragallia* is described and illustrated based on three males collected in Peru (Pasco Department). This is the second species of the genus recorded from Peru. The other recorded species is *E. prion* Kramer, 1976. A brief discussion about similarities and differences between the new species and *E. prion* is included. The new species has remarkable male genital features, including a peculiar structure connecting the subgenital plates to the styles, which is herein described.

Material and methods

The studied specimens belong to the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM; Lima), Illinois Natural History Survey (INHS; Champaign), and Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ; Rio de Janeiro). Label data are given inside quotations with a reversed virgule (\) separating lines. Morphological terminology follows mainly Oman (1933, 1938b), except for the head (Hamilton 1981, Mejdalani 1998) and leg chaetotaxy (Davis 1975). Techniques for preparation of male genital structures follow Oman (1949). The dissected parts are stored in microvials with glycerin. The photographs of the body and of the face were prepared with the Automontage software (Synoptics Inc., Frederick, Maryland, USA) using a digital camera attached to a stereomicroscope.

Results

Euragallia batmani Rodrigues, Gonçalves & Mejdalani, sp. n.

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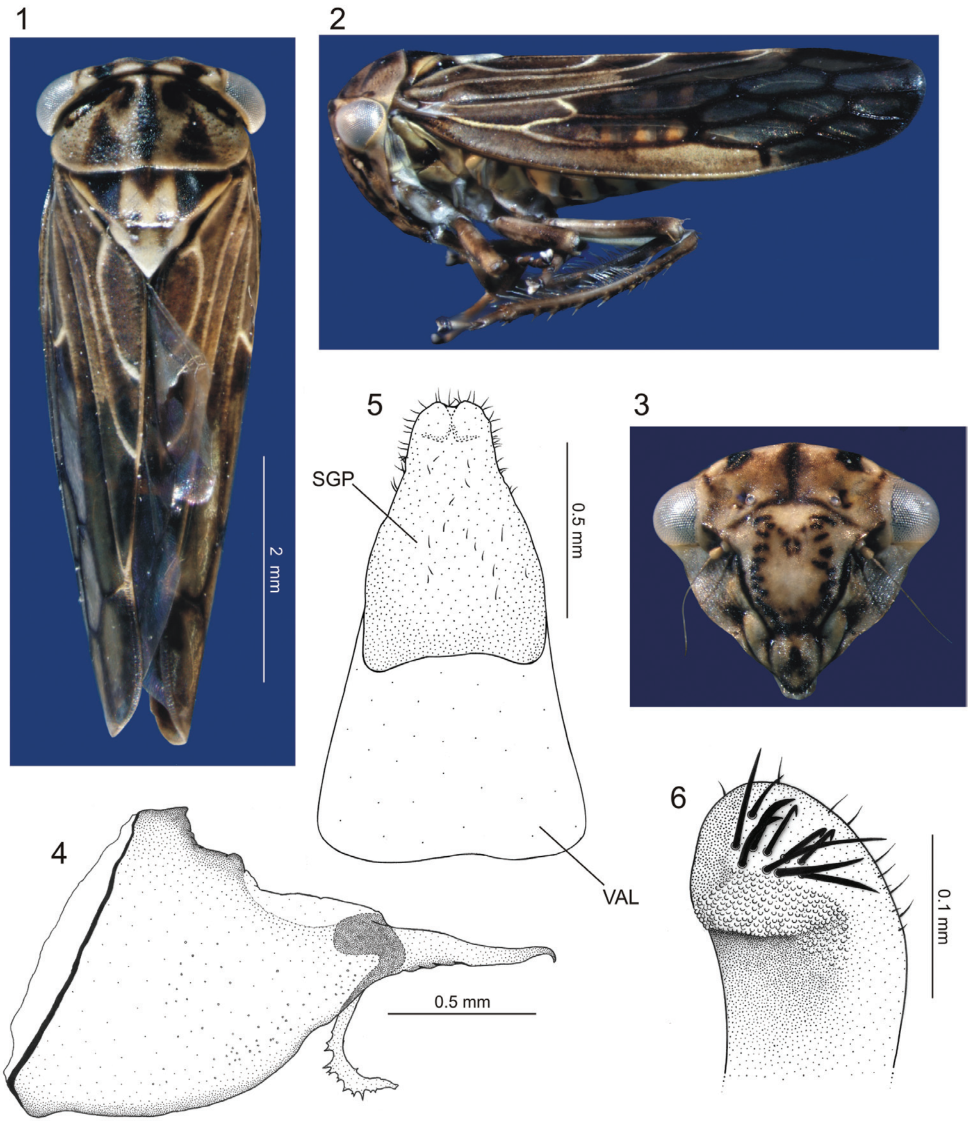
http://species-id.net/wiki/Euragallia_batmani
Figs 1–11

Length. Male holotype, 6.1 mm; male paratypes, 6.4–6.5 mm (n = 2).

Holotype description. Head and thorax. Ground color of dorsum mostly yellow to brown. Crown, in dorsal view (Fig. 1), distinctly shorter medially than next to eyes; each side with fovea closer to inner eye margin than to median coronal line, each fovea with adjacent black macula; median portion of crown with black spot. Face (Fig. 3) with distinct inverted Y-shaped dark brown macula; surface depressed medially; distance between ocelli greater than distance between each ocellus and adjacent inner eye margin; ocelli slightly closer to median line of face than to adjacent inner eye margin, each ocellus with adjacent fovea and small, inconspicuous dark brown macula. Frons (Fig. 3) mostly yellow; lateral margins bordered by black line; disk with somewhat M-shaped figure formed by numerous small dark brown maculae. Genae (Fig. 3) mostly yellow, with one dark brown macula adjacent to lorum and another adjacent to antennal base; surface striated. Clypeus (Fig. 3) with transverse dark brown line at base and median dark brown macula; with small apical setae. Pronotum (Fig. 1) with pair of black-marked depressions on anterior margin; disk weakly punctured and finely transversely striated; with pair of large dark brown triangular maculae on posterior half, dark brown longitudinal line extending from anterior to posterior margin on middle portion, and dark brown macula between apex of each triangular macula and median line. Basal portion of mesonotum (Fig. 1) with triangular dark brown macula located medially and pair of larger, lateral dark brown triangular maculae, these three maculae delimiting yellow, somewhat M-shaped area; pair of dark brown spots close to transverse sulcus. Forewings (Figs 1, 2) opaque on basal third and along costal area until base of first apical cell, remainder of surface translucent; venation pronounced; claval veins almost entirely marked with pale yellow; corium veins marked with pale yellow on basal third, remainder mostly dark brown; claval apex marked with pale yellow.

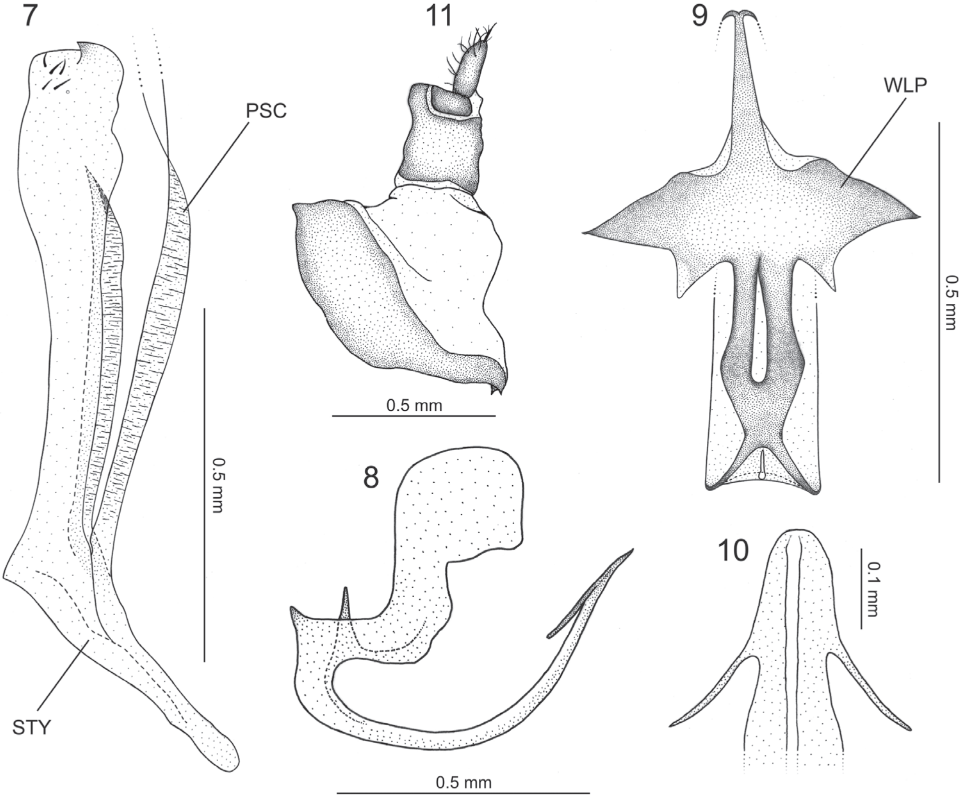
Metathoracic leg chaetotaxy. Row I of tibia with ten primary setae, equal in size and shape, bases slightly elevated. Row II with seven primary setae, bases elevated. Row III with eight primary setae, most proximal one almost aligned with third seta of row II. Row IV with approximately 25 primary elongate setae, increasing in size towards tibia apex; intercalary setae present; distal setae indistinct from the others of the row. Apex of



Figures 1–6. *Euragallia batmani* sp. n., body and external male genitalia. **1** body, dorsal view **2** body, lateral view **3** face, frontal view **4** pygofer, lateral view **5** valve (VAL) and (fused) subgenital plates (SGP), ventral view **6** apical portion of subgenital plate, laterodorsal view.

tibia with distal transverse row formed by conspicuous spurs. Plantar surface of first tarsomere with small setae uniformly distributed; apex bearing one conspicuous platella.

Male genitalia. Pygofer (Fig. 4), in lateral view, pronounced posteriorly, forming very long projection with about half of pygofer length; apex of projection hook-like; inner dorsal margin with elongate process extending ventrally and then gradually curved posteriorly, armed apically with irregular dentiform projections. Valve (Fig. 5) trapezoi-



Figures 7–11. *Euragallia batmani* sp. n., internal male genitalia and anal tube. **7** style (STY), dorsal view (PSC = plate-style connective) **8** aedeagus, lateral view **9** dorsal region of basal portion of aedeagus, dorsal view (WLP = wing-like projection) **10** apical portion of aedeagus, caudal view **11** anal tube, lateral view.

dal, fused to subgenital plates. The latter (Fig. 5), in ventral view, completely fused to each other, subtriangular, with slight apical emargination; surface with small scattered setae; apical portion, in lateral view, curved dorsally, with some well-developed setae and scale-like sculpture (Fig. 6). Connective membranous. Styles (Fig. 7), in dorsal view, expanded on apical third, club-shaped, bearing small apical claw-like projection directed inward; basal portion of style with elongate sclerotized projection, which is fused apically to the dorsal apical portion of subgenital plate and lays on a groove on outer style margin (Fig. 7 – PSC [*plate-style connective*]). Aedeagus (Fig. 8) symmetrical; shaft, in lateral view, strongly flattened dorsoventrally, base directed ventrally, then gradually curved dorsally; apical portion directed posterodorsally, bearing pair of subapical, slender lateral spiniform processes (Fig. 10) extending anteriorly for short distance; base of aedeagus, in dorsal view, with dorsal region well-developed, bearing lateral sclerotized projections resembling the open wings of a bat (Fig. 9 – WLP); base of aedeagus (Fig. 8), in lateral view, with spiniform projection directed dorsally. Segment X (Fig. 11), in lateral view, strongly sclerotized, bearing pair of small apical processes on each side.

Female unknown.

Intraspecific variation. The number of primary setae on the rows of the metathoracic tibia may vary slightly; for instance, the number of setae on row III may be eight (holotype) or nine (one paratype). Additional cross veins may be present on the forewings; for instance, the median anteapical cell may be divided by a cross vein. The basal portion of the forewings may be more translucent than in the holotype.

Etymology. The specific epithet, *batmani*, is a reference to the dorsal region of the aedeagal base in dorsal view (Fig. 9), which closely resembles the open wings of a bat, like those of the Batman symbol.

Type material. Peru, Pasco Department. Holotype: male, "Peru: Pasco, Yanachaga-Chemil- \ lén N.P., Huampal Stn. 10°11'9"S, 75°34'27"W, 1050m, 6–9 X 2002 \ D. Takiya, C. Peña, R. Rakitov \ Malaise trap acr. R. Huancabamba" (MUSM). Paratypes: two males with same data as the holotype (INHS, MNRJ).

Discussion

Among the *Euragallia* species, *E. prion* seems to be the most similar to *E. batmani* sp. n. In Gonçalves and Zanol (2010), the new species keys to the couplet of *E. prion*, and according to their proposal of species groups, it belongs in the *major* group, as well as *E. prion*. The male pygofer of both species has the posterodorsal process armed with similar dentiform projections. The two species also have a pair of lateral subapical processes on the aedeagal shaft. Nevertheless, the new species can be easily distinguished from *E. prion* by the shape of the posterior margin of the pygofer, which is very pronounced posteriorly in the former (Fig. 4). Such pygofer projection is absent in *E. prion*. The bat-like shape of the dorsal region of the aedeagal base (Fig. 9 – WLP) is another character that clearly distinguishes *E. batmani* from *E. prion* and the remaining species of the genus.

The basal portion of the styles of the new species bears a conspicuous, sclerotized elongate projection, which lays on a groove on the outer style margin. This projection, which is fused apically to the dorsal apical portion of the subgenital plate, is herein named the *plate-style connective* (Fig. 7 – PSC). This structure was previously recorded in only three species of the genus, *E. goemansi* Gonçalves & Zanol, 2010, *E. adelinae* Gonçalves & Zanol, 2010, and *E. nervata* (Oman, 1934), being described and illustrated in the first two by Gonçalves and Zanol (2010) and illustrated only in the last by Linnavuori and DeLong (1979). Despite the fact that the PSC is recorded in the literature for only three species, the present authors have noticed that the majority of the species within the genus possess this structure. We believe that future studies will point out the relevance of the PSC for the taxonomy of *Euragallia*. The function of the PSC is not known. It is also not known whether the development of the PSC is morphofunctionally associated with the reduced (membranous) connective of the new species or whether the two conditions arose independently.

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Diversity of the strongly rheophilous tadpoles of Malagasy tree frogs, genus *Boophis* (Anura, Mantellidae), and identification of new candidate species via larval DNA sequence and morphology

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Abstract

This study provides detailed morphological descriptions of previously unknown tadpoles of the treefrog genus *Boophis* Tschudi and analyses of habitat preferences of several of these tadpoles in Ranomafana National Park. A total of twenty-two tadpoles determined via DNA barcoding are characterized morphologically herein, fourteen of them for the first time. Twelve of these tadpoles belong to taxonomically undescribed candidate species which in several cases are so far only known from their larval stages. Our data show that the larvae of some of these candidate species occur syntopically yet maintaining a clearly correlated genetic and morphological identity, suggesting that they indeed are true biological and evolutionary species. Tadpoles considered to belong to the "adherent" ecomorphological guild inhabit fast-running waters and their oral disc is commonly to continuously attached to the rocky substrate, supposedly to keep their position in the water current. Some of these species are characterized by the presence of a dorsal gap of papillae and the absence of an upper jaw sheath. This guild includes the tadpoles of the *B. albipuncatus* group (*B. ankaratra*, *B. schuboeae*, *B. albipunctatus*, *B. sibilans*, *B. luciae*), and of the *B. mandraka* group (*B. sambirano* and six candidate species related to this species and to *B. mandraka*). Tadpoles considered belonging to the "suctorial" guild inhabit fast-running waters where they use frequently their oral disc to attach to the substrate. They have an enlarged oral disc without any dorsal gap, including two nominal species (*B. marojezensis, B. vittatus*), and five candidate species related to *B. marojezensis.* An ecological analysis of the tadpoles of *B. luciae*, *B. schuboeae* and *B. marojezensis* [Ca51 JQ518198] from Ranomafana National Park did not provide evidence for a clear preference of these tadpoles to the fast flowing microhabitat sections of the stream, although the tadpoles discussed in this study are typically caught in this habitat.

Keywords

Amphibia, Anura, Mantellidae, *Boophis*, larval morphology, oral disc, clasping, adherent, suctorial, candidate species, larval ecology, microhabitat preference

Introduction

The genus *Boophis* Tschudi, 1938 is a species-rich group of treefrogs in the family Mantellidae which is endemic to Madagascar and to the Comoran island of Mayotte. Seventy-two nominal species and over 25 candidate species of *Boophis* are currently known (Vieites et al. 2009, Vallan et al. 2010, Glaw et al. 2010, Vences et al. 2010a, b).

Tadpoles have been described for 46 species of *Boophis* (e.g., Blommers-Schlösser 1979, Thomas et al. 2005, Grosjean et al. 2006, Raharivololoniaina et al. 2006, Altig and McDiarmid 2006, Glos et al. 2007, Schmidt et al. 2008, Randrianiaina et al. 2009a, b, Rasolonjatovo et al. 2010). Compared to many other Malagasy anuran groups, the larval stages of *Boophis* are therefore quite well known, possibly because they are relatively easy to find in rainforest streams (e.g., Strauß et al. 2010) and even sometimes outside the forest (Glaw and Vences 2007).

The existence of strongly rheophilous tadpoles in species of *Boophis* has been known since the work of Blommers-Schlösser (1979). This author pioneered our understanding of the evolutionary relationships and natural history of Malagasy frogs and described several tadpoles with peculiar morphological characteristics such as an enlarged oral disc, increased number of keratodont rows and papillae, and low tail fin. These larvae were assigned to *Boophis majori, B. erythrodactylus*, *B. mandraka*, and *Boophis* sp.. However, in 1979 the true species diversity of *Boophis* was not fully understood (Glaw et al. 2001) and matching of tadpoles to species was difficult without molecular genetic techniques, which resulted in equivocal identity of the tadpoles from these early studies. Tadpoles assigned by Blommers-Schlösser (1979) to *Boophis majori* probably belong to *B. marojezensis*, and the identity of tadpoles assigned to *B. erythrodactylus* remains uncertain, because this species belongs to a species group which has generalized tadpoles. Subsequent to these early works, Raharivololoniaina et al. (2006) described the tadpoles of *B. marojezensis* and *B. sibilans* from Andasibe. Glos et al. (2007) described *B. schuboeae* tadpoles from Ranomafana and *B. ankaratra* tadpoles from Andringitra, and Thomas et al. (2006) described *B. andohahela* tadpoles from Ranomafana. More recently, Rasolonjatovo et al. (2010) described the larvae of *B. englaenderi*, *B. luciae,* and *B. vittatus*.

In this study, morphological data on twenty-two strongly rheophilous tadpoles are provided, of which fourteen were previously unknown. Twelve of these larvae belong to candidate species which so far have not been scientifically named.

All these strongly rheophilous tadpoles are characterized by their "streamlined" (i.e., elongated, narrow and flat) body form, their wide oral disc containing many keratodont rows with all posterior rows uninterrupted, their completely keratinized jaw sheaths, of which the lower one is always "ribbed" and the upper one can be absent in some species, and rows of many small rounded marginal papillae with or without a dorsal gap. The absence of many of these characteristics in *B. williamsi* tadpoles (Blommers-Schlösser 1979 and Schmidt et al. 2008) is the criteria of excluding them from the present study.

In the context of grouping Malagasy tadpoles into different ecomorphological guilds, some *Boophis* tadpoles have been classified as "suctorial" and "adherent" by Altig and Johnston (1989), and Raharivololoniaina et al. (2006) have classified other *Boophis* tadpoles more in detail according to their morphological characters. However, for a definition of ecomorphological guilds, it is appropriate to include also ecological data. Therefore, we here combine our morphological descriptions with the results of an ecological analysis of the three most abundant strongly rheophilous tadpoles in Ranomafana National Park in the southern central east of Madagascar (*B. luciae*, *B. marojezensis* [Ca51], and *B. andohahela*). Based on habitat characteristics from 30 streams in this rainforest reserve we tested whether the typical morphological characteristics of these tadpoles are indeed associated with a preference for a fast-running stream habitat, as it has been predicted by Blommers-Schlösser (1979).

The use of DNA barcoding to identify amphibian larvae from species-rich tropical communities to the species level has been tremendously successful within the last years (e.g., Thomas et al. 2005). It also has induced a so called "reverse taxonomy" in this vertebrate group (Randrianiaina et al. 2011a), with species new to science discovered first by their larvae rather than their adult stage. The present study confirms this progress by discovering twelve candidate species via tadpole DNA sequences and morphology, many of which are still unknown in their adult stage.

Materials and methods

Morphological study of tadpoles

Tadpoles were collected by different kinds of nets having mesh sizes from 2 to 5 mm, depending on the size of the streams, the strength of the water current and the type of substrate. They were euthanised by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters (body shape, relative tail length, eye position and direction, oral disc position, direction and configuration, general color pattern). From each series one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. This specimen is here called "DNA voucher". All detailed morphological tadpole characterizations and drawings are based on this DNA voucher, whereas variation is sometimes described based on further specimens of the series. After tissue collection, all specimens were preserved in 5% formalin or 70% ethanol. Specimens were deposited in the Zoologische Staatssammlung München, Germany (ZSM). When referring to voucher specimens the original field numbers (FG/MV, FGZC, T, and ZCMV) are usually provided together with the final ZSM catalogue numbers. Tadpoles studied in this paper are summarized in Tables 1 and 2, including data concerning the site and its coordinates, the date of the capture and the collectors.

For detailed morphological examination, especially to determine developmental stages and assess characters of the oral disc, preserved tadpoles were stained slightly with methylene blue. Tadpoles were examined under water, and a few drops of methylene blue were applied to the oral disc, hind limb, spiracle, narial opening and vent tube to better discern their structures. Developmental stages were determined following Gosner (1960).

Morphological descriptions, measurements and drawings were done on digital pictures of the preserved tadpoles taken with a stereomicroscope Zeiss StereoDiscovery V12 connected to a computer, following landmarks, terminology and definitions of Altig and McDiarmid (1999) and Randrianiaina et al. (2011a), except that we predominantly use the term keratodonts instead of labial teeth. The formula of keratodont rows (labial tooth row formula, LTRF) is given according to Altig and McDiarmid (1999). Comparing measurements, we consider them as "almost equal" if ratios of the measured values are 95–96% or 104–105%, "equal" if they are in the range 97–103%, as "almost in the middle" if they are in the range 45–46% or 54–55% and "in the middle" if they are in the range 47–53% (Randrianiaina et al. 2011a). All the measurement data are summarized in Tables 3 to 5 electronic supplement. Most of these data, especially concerning the oral disc, are used for elaborating morphological clusters, into which all tadpoles are classified. The following abbreviations are used: A_1 (first upper keratodont row), A_2 (second upper keratodont row), A_{2gap} (medial gap in A_2), A_3 (third upper keratodont row), A_4 (fourth upper keratodont row), A_5 (fifth upper keratodont row), A_6 (sixth upper keratodont row), A_7 (seventh upper keratodont row), A_8 (eighth upper keratodont row), $A_{1-8\ den}$ (density of the keratodonts in row A_{1-8}), $A_{1-8\ len}$ (length of A_{1-8}), $A_{1-8\ num}$ (number of keratodonts in A_{1-8}), BH (maximal body height), BL (body length), BW (maximal body width), DF (dorsal fin height at midtail), DG (size in rows of the dorsal gap of marginal papillae), DMTH (distance of maximal tail height from the tail-body junction), ED (eye diameter), EH (eyes height – measured from the lower curve of the belly), HAB (height of the point where the axis of the tail myotomes contacts the body – measured from the lower curve of the belly), IND (inter-narial distance), IOD (inter-orbital distance), JW (maximal jaw sheath width), MC (medial convexity of the upper sheath), MCL (length of the medial convexity of the upper sheath), MP (marginal papillae), MTH (maximal tail height), ND (naris diameter), NH (naris height - measured from the lower curve of the belly), NP (naris-pupil distance), OD (oral disc), ODW (maximum oral disc width), P_1 (first lower keratodont row), P_2 (second lower keratodont row), P_3 (third lower keratodont row), $P_{1-3\ den}$ (density of the keratodonts in P_{1-3}), $P_{1-3\ len}$ (length of P_{1-3}), $P_{1-3\ num}$ (number of keratodonts in P_{1-3}), PCA (Principal Component Analysis), RN (rostro-narial distance), SBH (distance between snout and the point of maximal body height), SBW (distance between snout and the point of maximal body width), SE (snout-eye distance), SH (spiracle height – measured from the lower curve of the belly), SL (spiracle length), SMP (submarginal

Table 1. Summary of localities with geographic coordinates, and collection dates, of tadpole specimens studied herein.

Locality	Site	Species	Coordinates	Date	Collectors
Ankijagna Lalagna		*B. sambirano* [Ca49]	14°14.055'S 48°58.732'E 1187 m a.s.l.	08.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina
Ambohitsara		*B. albipunctatus*	21°21.431'S 47°48.941'E 294 m a.s.l.	03.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Ambinanitelo		*B. marojezensis* [Ca52]	14°13.524'S 48°57.808'E 1182 m a.s.l.	09.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina
Ambinanitelo		*B. sambirano* [Ca50]	14°13.524'S 48°57.808'E 1182 m a.s.l.	09.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina
An'Ala	Andohanisity	*B. mandraka* [Ca46]	18°55.156'S 48°29.278'E 889 m a.s.l.	08.02.2006	C. Patton, D.R. Vieites, J. Patton, L. Raharivololoniaina, M. Vences, R.D. Randrianiaina
Andasibe Special Reserve	Analamazaotra river	*B. sibilans*	18°55.900'S 48°25.733'E 900 m a.s.l.	04.12.2001	L. Raharivololoniaina, M Vences
Between Antsohihy and Bealanana	Anjingo river	*B. sambirano* [Ca47]	14°44.929'S 48° 29.491'E 925 m a.s.l.	07.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina
Between Antsohihy and Bealanana	Anjingo river	*B. sambirano* [Ca48]	14°44.929'S 48°29.491'E 925 m a.s.l.	07.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina
Manongarivo Special Reserve	Camp Norbert	*B. sambirano*	13°56.053'S 48°27.028’E 288 m a.s.l.	31.01.2003	F. Glaw, M. Vences, R.D. Randrianiaina
Marojejy National Park	Camp Mantella	*B. vittatus*	14°26.972'S 49°47.214'E 327 m a.s.l.	14.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina
Marojejy National Park	Camp Marojejia	*B. englaenderi*	14°26.070'S 49°45.638'E 740 m a.s.l.	18.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina

Locality	Site	Species	Coordinates	Date	Collectors
Marojejy National Park	Camp Mantella	*B. englaenderi* [Ca23]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina
Marojejy National Park	Camp Mantella	*B. marojezensis* [Ca25]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina
Marojejy National Park	Camp Mantella	*B. marojezensis* [Ca26]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina
Marojejy National Park	Camp Mantella	*B. sibilans*	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiaina
Ranomafana National Park	Ambatolahy river	*B. andohahela*	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiaina
Ranomafana National Park	Ambatolahy river	*B. marojezensis* [Ca51]	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiaina
Ranomafana National Park	Ambatolahy river	*B. schuboeae*	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiaina
Ranomafana National Park	Imaloka	*B. marojezensis* [Ca51]	21°14.529'S 47°27.938'E 957 m a.s.l.	01.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Ranomafana National Park	In a pool below waterfall	*B. schuboeae*		11.02.2003	M. Teschke, M. Vences
Ranomafana National Park	Marihy avaratra	*B. luciae*	21°15.806'S 47°25.548'E 1144 m a.s.l.	20.02.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Ranomafana National Park	Marihy avaratra	*B. mandraka* [Ca38]	21°15.806'S 47°25.548'E 1144 m a.s.l.	02.02.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Ranomafana National Park	Talatakely	*B. luciae*	21°15.846'S 47°25.161'E 966 m a.s.l.	24.02.2006	L. Raharivololoniaina, A.F. Ranjanaharisoa, T.J. Razafindrabe, D.R. Vieites, J. Patton, C. Patton, M. Vences, R.D. Randrianiaina
Ranomafana National Park	Talatakely	*B. marojezensis* [Ca51]	21°15.846'S 47°25.161'E 966 m a.s.l.	24.02.2006	L. Raharivololoniaina, A.F. Ranjanaharisoa, T.J. Razafindrabe, D.R. Vieites, J. Patton, C. Patton, M. Vences, R.D. Randrianiaina

Locality	Site	Species	Coordinates	Date	Collectors
Ranomafana National Park	Sahateza (Pond Donald)	*B. ankaratra*	21°15.476'S 47°21.583'E 1016 m a.s.l.	03.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Ranomafana National Park	Vatoharana	*B. andohahela*	21°17.338'S 47°25.765'E 1016 m a.s.l.	24.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiaina
Tsaratanana Strict Nature Reserve	Antevialambazaha	*B. marojezensis* [Ca53]	14°10.455'S 48°56.714'E 1699 m a.s.l.	10.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiaina

Table 2. Collection numbers and Genbank accession numbers of the tadpoles studied. FG/MV, FGZC, LR, T, TAD, ZCMV (field numbers), ZSM (Zoologische Staatssammlung München). Missing accession numbers indicate that sequences were too short or of poor quality and were therefore not submitted to Genbank, or that they will be submitted to Genbank in the course of future studies.

Species	Locality	ZSM- and Field number	Accession number
Boophis englaenderi	Marojejy National Park	FGZC 2244- ZSM 623/2008	HM769921
B. englaenderi [Ca23]	Marojejy National Park	FGZC 2957- ZSM 1632/2007	JQ518193
		FGZC 2241- ZSM 1499/2007	---
		FGZC 2243- ZSM 527/2008	FJ559144
		FGZC 2248- ZSM 1508/2007	---
		FGZC 2250- ZSM 1502/2007	---
		FGZC 2252- ZSM 1503/2007	---
		FGZC 2257- ZSM 529/2008	---
		FGZC 2260- ZSM 530/2008	---
		FGZC 2273- ZSM 1514/2007	---
		FGZC 2275- ZSM 1516/2007	---
Boophis andohahela	Ranomafana National Park	T 60- ZSM 912/2007	GU974437
		T 107- ZSM 1319/2007	GU974422
		T 125- ZSM 1321/2007	GU974423
		T 127- ZSM 1162/2007	GU974424
		T 131- ZSM 1351/2007	GU974425
		T 150- ZSM 910/2007	GU974427
		T 222- ZSM 566/2007	GU974435
		T 428- ZSM 998/2007	GU974449
		T 09/273- ZSM 282/2009	---
Boophis ankaratra	Ranomafana National Park	FGMV 2003.1698- ZSM 816/2004	---
		ZCMV 3803- ZSM 168/2008	---
		ZCMV 4917- ZSM 876/2007	GU974476

Species	**Locality**	**ZSM- and Field number**	**Accession number**
Boophis schuboeae	Ranomafana National Park	FGMV 2002.1800- ZSM 978/2004	DQ068394
		Tad 2004-780- ZSM 1339/2004	---
		Tad 2004-797- ZSM 1356-2004	---
		T 09/980- ZSM 743/2008	---
		T 09/968- ZSM 739/2008	---
		T 09/971- ZSM 740/2008	---
		T 09/998- ZSM 749/2008	---
B. albipunctatus	Ambohitsara	ZCMV 4942- ZSM 78/2008	GU974373
		ZCMV 4946- ZSM 82/2008	GU974374
Boophis sibilans	An'Ala	ZCMV 3450- ZSM 1754/2007	---
Boophis sibilans	Andasibe	LR 269- ZSM 557/2004	DQ792492
Boophis sibilans	Marojejy National Park	FGZC 2956- ZSM 1631/2007	JQ518194
Boophis luciae	Ranomafana National Park	T 176- ZSM 792/2007	---
		T 177- ZSM 593/2007	GU975090
		T 178- ZSM 541/2007	GU975094
		T 179- ZSM 976/2007	---
		T 224- ZSM 264/2007	---
		T 430- ZSM 274/2007	GU975096
		ZCMV 3619- ZSM 1587/2006	HM769939
		ZCMV 3631- ZSM 1588/2006	HM769940
		ZCMV 3686- ZSM 634/2008	HM769938
		ZCMV 4024- ZSM 688/2007	---
		ZCMV 5146- ZSM 730/2007	---
B. sambirano	Manongarivo Special Reserve	FGMV 2002.1904- ZSM 678/2004	EU717863
		FGMV 2002.1902- ZSM 672/2004	EU717861
B. mandraka [Ca38]	Ranomafana National Park	ZCMV 4261- ZSM 456/2007	FJ559153
B. mandraka [Ca46]	An'Ala	ZCMV 3479- ZSM 1784/2007	JQ518195
B. sambirano [Ca47]	Between Antsohihy and Bealanana	ZCMV 13105- ZSM 482/2010	JQ518203
		ZCMV 13110- ZSM 486/2010	JQ518204
B. sambirano [Ca48]	Between Antsohihy and Bealanana	ZCMV 13107- ZSM 484/2010	JQ518206
		ZCMV 13108- ZSM 485/2010	JQ518207
		ZCMV 13109- ZSM 485/2010	JQ518205
B. sambirano [Ca49]	Ankijagna Lalagna	ZCMV 13150- ZSM 523/2010	JQ518209
		ZCMV 13155- ZSM 528/2010	JQ518208
		ZCMV 13156- ZSM 529/2010	JQ518210
B. sambirano [Ca50]	Ambinanitelo	ZCMV 13171- ZSM 544/2010	JQ518212
		ZCMV 13172- ZSM 545/2010	JQ518211
		ZCMV 13173- ZSM 546/2010	JQ518213
		ZCMV 13174- ZSM 547/2010	JQ518214
B. marojezensis	Marojejy National Park	FGZC 2277- ZSM 1528/2007	JQ518196
		FGZC 2953- ZSM 1628/2007	JQ518199
B. marojezensis [Ca25]	Marojejy National Park	FGZC 2929- ZSM 1611/2007	FJ559146
B. marojezensis [Ca26]	Marojejy National Park	FGZC 2930- ZSM 1612/2007	JQ518197
B. marojezensis [Ca51]	Ranomafana National Park	T 394- ZSM 1008/2007	GU974657
		T 432- ZSM 117/2007	GU974658
		T 09/1088- ZSM 779/2008	---

Species	**Locality**	**ZSM- and Field number**	**Accession number**
		T 09/1091- ZSM 780/2008	---
		T 09/1094- ZSM 781/2008	---
		ZCMV 3691- ZSM 267/2008	---
		ZCMV 3629- ZSM 318/2008	---
		ZCMV 3635- ZSM 232/2008	---
		ZCMV 3690- ZSM 266/2008	---
		ZCMV 3742- ZSM 481/2008	---
		ZCMV 4203- ZSM 401/2007	---
		ZCMV 4264- ZSM 457/2007	GU974654
		ZCMV 4376- ZSM 1453/2007	GU974647
		ZCMV 4531- ZSM 532/2007	GU974648
		ZCMV 4541- ZSM 504/2007	GU974650
		ZCMV 4547- ZSM 1390/2007	GU974651
		ZCMV 4550- ZSM 509/2007	GU974652
		ZCMV 4931- ZSM 838/2007	GU974656
		ZCMV 5098- ZSM 913/2007	GU974646
		ZCMV 5986- ZSM 1212/2007	GU974655
		ZCMV 1395- ZSM 0025/2007	GU974653
		T 09/1085- ZSM 778/2008	---
B. marojezensis [Ca52]	Ambinanitelo	ZCMV 13168- ZSM 541/2010	JQ518215
		ZCMV 13169- ZSM 542/2010	---
B. marojezensis [Ca53]	Tsaratanana Strict Nature Reserve	ZCMV 13200- ZSM 573/2010	JQ518216
		ZCMV 13201- ZSM 574/2010	---
		ZCMV 13202- ZSM 575/2010	---
		ZCMV 13203- ZSM 576/2010	---
		ZCMV 13204- ZSM 577/2010	---
		ZCMV 13205- ZSM 578/2010	JQ518217
Boophis vittatus	Marojejy National Park	FGZC 2237- ZSM 5219/2005	---
		FGZC 2238- ZSM 1906/2007	JQ518200
		FGZC 2251- ZSM 1907/2007	JQ518201
		FGZC 2914- ZSM 1601/2007	JQ518202

papillae), SS (snout-spiracle distance), SV (spiracle-vent distance), TAL (tail length), TH (tail height at the beginning of the tail), THM (tail height at midtail), Thorn-pap (thorn-shaped papillae), TL (total length), TMH (tail muscle height at the beginning of the tail), TMHM (tail muscle height at midtail), TMW (tail muscle width at the beginning of the tail), LR (number of the lower rows of keratodonts), UR (number of the upper rows of keratodonts), VF (ventral fin height at midtail), VG (size in rows of the ventral gap of marginal papillae), VL (vent tube length).

In tadpoles of many frog species, pigmentless parts of the body wall become detached and apparently separated by a liquid-filled cavity from the underlying pigmented parts of the skin and the inner organs (among Malagasy frogs, for instance extremely expressed in the tadpoles of some *Scaphiophryne*; see Grosjean et al. 2007). These cavities probably represent lymphatic sacs or sinuses but this hypothesis has not been verified in most anuran species. In the rheophilous *Boophis* tadpoles, the extension of this detachment of a transparent part of the body wall appears to be characteristic for some species and candidate species. The difference often refers to the extension and ease to recognize this transparent area of the body wall, and we therefore use terms like recognizable vs. poorly recognizable rather than present vs. absent when referring to this structure, although there are clearly pronounced differences in its expression among some species.

DNA-based species identification

DNA barcoding was based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently variable among species of Malagasy frogs (Vences et al. 2005). We amplified a fragment of ca. 550 bp using primers 16Sar-L and 16Sbr-H from Palumbi et al. (1991), or a shorter fragment of ca. 400 bp using the newly developed specific mantellid primers 16S-Frog-L1 (CAT AAT CAC TTG TTC TTT AAA) and 16S-Frog-H1 (GAT CCA ACA TCG AGG TCG). PCR was carried out with standard protocols (Vences et al. 2005) and sequences resolved on automated sequencers. Sequences were preliminarily identified using BLAST searches against a near-complete database of sequences of adult Malagasy frog species. Results were subsequently verified by manually aligning and comparing sequences to the closest hits in the data base. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and clearly less similar to all sequences from other species. When no identity with adult specimens was found and divergence was >3% we considered the corresponding tadpoles to belong to undescribed candidate species. Newly determined DNA sequences were deposited in Genbank (accession numbers JQ518193- JQ518217).

Candidate species nomenclature followed the scheme developed by Padial et al. (2010). We use the binomial species name of the closely related species, followed in square by the abbreviation “Ca” with an attached numerical code referring to the particular candidate species, and at first mention terminating with the author name and the year of publication of the article in which the lineage was first discovered for few species, or the Genbank accession number of a DNA sequence of a reference specimen for others. Further in the text, we abbreviate the candidate species name just by using the binomial species name followed in square brackets by the abbreviation “Ca” and its numerical code.

Ecological study of tadpoles

During a study on stream tadpole communities in Ranomafana National Park (RNP) in the south eastern escarpment of Madagascar, we exhaustively sampled 33 stream sections for tadpoles (Strauß et al. 2010). Each section spanned 30 m and the sampling process was conducted separately for all available microhabitats within the section. We aimed to exhaustively sample tadpoles using dip nets of different sizes and materials, adjusted to obtain optimal sampling results for each stream. Sampling started downstream, and depending on stream width two to five people processed slowly on the same level upstream while dip-netting as much as reasonably possible all tadpoles in all microhabitats. These microhabitats were predefined subject to underground substrate (rock, gravel, leaves, sand) as well as separately by the stream velocity categories "fast" (obviously running) and "slow" (almost stagnant). Habitat variables were recorded at two spatial levels: *(1)* habitat variables of possible importance for breeding site (stream) choice of frog species and *(2)* proportion of microhabitats available within the streams.

We used data from this study for an exemplary analysis of breeding site choice and microhabitat use of syntopic species of strongly rheophilous tadpoles. To identify the habitat variables of the stream and the surrounding forest that may be important for breeding site choice, we performed a principal component analysis (PCA) and plotted species according to their incidence as supplementary variables in the PCA biplot. For PCA, we used all ten habitat variables of all 33 streams sampled during the tadpole community study. PCA was run on the correlation matrix in order to standardize for the influence of unequal variance. To evaluate data outliers and linear interdependence of variables, box-plots and pair-plots (Zuur et al. 2007) were used. As PCA requires multinormality of data, box-cox-power-transformations (Box and Cox 1964) were applied when necessary. The number of meaningful PCs was estimated by a scree plot (Zuur et al. 2007). PCA and correlation with species incidence was evaluated using the dimdesc function in package FactoMineR (Lê et al. 2008).

To analyze the use of microhabitats within streams, we first constructed graphs of raw data to display the species specific distribution between microhabitats. In order to quantify true preferences for microhabitats, Ivlev's electivity index (E, Ivlev 1961) was calculated for each strongly rheophilous *Boophis* species occurring in RNP. E is defined as E=(r-p)/(r+p) with r being the proportions of the microhabitats used and p the proportion of microhabitats available. To test whether the E values differ for the single species, a factorial ANOVA was run with E as dependent variable and the factors "microhabitat" and "species" as independent variables. This provides information whether E is different for the different microhabitats, whether E differs between species, and, if interactions could be included in model, whether the effect of the one factor depends on the level of the other factor. To avoid possible overparameterisation caused by large numbers of interactions (Crawley 2007), we removed the interaction term from the model and performed ANOVAs of subsets of the data to closer evaluate differences in preferences between species within specific microhabitats (interactions). Only the three abundant species were included in this analysis. Also, for each species only streams with at least eight specimens of the respective species were included in the analysis to reduce the influence of many high avoidance values due to a general low number of tadpoles in a stream.

Statistical analysis were performed in R 2.9.2 (R Development Core Team 2009) including libraries car (Fox et al. 2008) and FactoMineR (Lê et al. 2008).

Results

Tadpole descriptions

We here provide a summary of the most important morphological characteristics of one representative species per species group, and brief accounts for all other species and candidate species in which we mainly emphasize their difference to the species described more completely, or to other species belonging to the same group. Standardized, detailed descriptions and assessments of variation for all species and candidate species are found in the electronic supplement. Original measurements and ratios are given in Tables 3–5 which are equally included as electronic supplement.

Boophis luteus group

This group is characterized by tadpoles having a generalized oral disc without lateral emargination and ventral gap of papillae, but the dorsal gap is wide to very wide. The anterior margin of the oral disc is a continuation of the snout. Usually A_1 is uninterrupted and P_1 is interrupted, except the three species described herein which are the ones in the group having the strongest expression of adaptations that we interpret as rheophilous. The jaw sheaths are very strong with smooth surface and completely or partially keratinized in some species. The upper sheath is always made up by a medial convexity. Dorsolateral glands which exist in some other *Boophis* tadpoles are absent.

Boophis englaenderi Glaw & Vences, 1994

Morphological data were assessed in one tadpole (Figures 2 and 3) in developmental stage 36 (field number FGZC 2244; ZSM 623/2008, BL 11.8 mm, TL 25.4 mm, accession number HM769921) from Marojejy National Park (previously described by Rasolonjatovo Hiobiarilanto et al. 2010). The 16S rDNA sequence of this specimen is 99.5% identical to a reference sequence of an adult *B. englaenderi* (accession FJ559124) from Marojejy.

The tadpoles of this species have an elliptical body, a flatly rounded snout in dorsal view and a short tail. The distance between eyes is wide and nares are very large, round, positioned very high dorsally, and situated nearer to snout than to eye and at eye level. LTRF is 6(3–6)/3(1). The upper jaw sheath is totally keratinized with rounded serrations, moderately wide with a very short widely rounded medial convexity. The lower sheath is V-shaped, completely keratinized and partially hidden by the upper one. Both jaw sheaths have a smooth surface.

In preservative, the tadpole is generally dark brown. Dark brown spots condensed to form a hexagonal mark above the neurocranium; a dark semicircular patch situated posterior to each narial opening and dark patches between the vertebral area and the abdominal region are present. The snout is spotted. The transversal lines between the vertebral area and the abdominal region are perceivable which make the domino-like structure on this noticeable. The dorsal part of the tail muscle has five dark brown and four light alternating bands. The prominent dark brown band is the extension of the patches between the vertebral area and the abdominal region. The myosepta are visible on the dorsal part of the tail. Laterally, the jugal area is covered by dense dark brown patches and the dorsolateral part of the flank is identical to the dorsal pattern; the ventrolateral part is pale and the abdominal region is very dark leaving an opaque discernible spiracle. Ventrally, oral disc, gular and branchial regions are pale; the venter is more or less transparent and the intestinal coils are perceptible with a regularly spiral shape. The tail musculature is pale and covered by dark brown spots which condense to form reticulations. Fins are transparent, with few brown spots on the dorsal fin, and the ventral fin is free from pigment.

Boophis englaenderi **[Ca23 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 4) in developmental stage 30 (field number FGZC 2957, ZSM 1632/2007, BL 10.5 mm, TL 29.5 mm, Genbank accession number JQ518193) from Marojejy National Park. The 16S rDNA sequence of this specimen is 94% identical to a reference sequence of an adult *B. englaenderi* (accession AY848474) from Ilampy. Nine other voucher specimens agree in morphology with the voucher specimen described herein.

The external morphology of this tadpole has a very close similarity with that of *B. englaenderi*, except that it has a distinctly longer tail (TAL/BL 183% *vs.* 153%) and a lighter pigmentation. Additional differences between the two tadpoles are found in the oral disc structure. It is bulged laterally and has one more interrupted upper keratodont row and a first uninterrupted lower row giving the keratodont row formula LTRF 7(3–7)/3 *vs.* 6(3–6)/3(1). The number of papillae is higher than in *B. englaenderi* with 175 marginal papillae (*vs.* 128), and 94 submaginal papillae (*vs.* 33), although the examined tadpole is still in a developmental stage inferior to that of the examined tadpole of *B. englaenderi*. The submarginal papillae are complete on the lower labium. This tadpole is also characterized by a light brown coloration in preservative. The jugal area is covered by scarce light brown patches, and the tail musculature is covered by light brown spots which group in some areas to form patches or sparse reticulations. The intestinal coils are visible. The examination of nine other voucher specimens (see Table 2) confirms the differences to *B. englaenderi*.

Boophis andohahela **Andreone, Nincheri & Piazza, 1995**

Morphological data were assessed in one tadpole (Figures 2 and 5) in developmental stage 26 (field number T 428; ZSM 998/2007, BL 11.8 mm, TL 25.4 mm, Genbank accession number GU974449) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. andohahela* (accession AY848456) from the same locality. Five out of six other voucher specimens have the morphological characteristics of this species, whereas one tadpole has a difference in the oral disc configuration.

The general morphology of this tadpole is similar to that of *B. englaenderi* and *B. englaenderi* [Ca23], but it is characterized by the presence of a white patch posterior to the hexagonal mark above the neurocranium in life and even in preservative (Figure 1). The non-visibility of its intestinal coils is shared with *B. englaenderi*. The LTRF 6(3–6)/3 is identical to that of some specimens of *B. englenderi* but differs from that of *B. englaenderi* [Ca 23]. On the other hand, the absence of papillae on the ventral area of the lower labium is similar to that of *B. englaenderi*. The oral disc of this tadpole has a slightly developed lateral bulge.

Boophis albipunctatus group

This group is characterized by tadpoles having an enlarged oral disc without lateral emargination (but bulged laterally in some species) and ventral gap of marginal papillae. The dorsal gap is moderately wide. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the entire margin is free from the snout. LTRF 8(5–8)/3 or 7(5–7)/3. The jaw sheaths are moderately strong and completely keratinized. The upper sheath has a medial convexity in some species. The lower sheath is U or V-shaped and ribbed. Dorsolateral glands are present.

Boophis ankaratra Andreone, 1993

Morphological data were assessed in one tadpole (Figures 2 and 6) in developmental stage 28 (field number ZCMV 4917, ZSM 876/2007, BL 11.3 mm, TL 25.5 mm, Genbank accession number GU974476) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. ankaratra* (accession AJ315909) from Mandraka. Two other voucher specimens possess the typical morphological characters of the species.

This tadpole can be differentiated from *B. luteus* goup tadpoles by the general state of the oral disc. It is characterized by an enlarged and laterally bulged oral disc. There is a double row of marginal papillae interrupted by a moderately wide dorsal gap. Papillae are short, small, conical with protuberance, and their tip is rounded. There are 148 and 190 marginal and submarginal papillae, respectively. The LTRF is 8(5–8)/3 and A_1 is moderately long. The jaw sheaths are moderately strong and totally keratinized. The upper sheath is characterized by a short narrowly pointed medial convexity. The lower sheath is U-shaped, ribbed, higher than wide, and partially hidden by the upper one.

In life this tadpole is generally dark brown. Dorsally, body and tail covered by dense brown spots. A hexagonal mark above the neurocranium and a dark semicircular patch posterior to each narial opening are obvious. The domino-like structures between the vertebral area and the abdominal region are recognizable. Few irregular dark blotches and silvery spots scattered on the skin. Laterally, jugal area is covered by dense brown patches and the abdominal region is very dark leaving a transparent noticeable spiracle. The tail musculature is yellowish and covered by sparse brown spots which coalesce to form patches. Their density diminishes toward the tail tip. Fins are transparent with few brown blotches on the dorsal fin and the ventral fin is almost free from pigment. Ventrally, intestinal coils are invisible (Figure 1). In preservative, the tadpole is similar except that it is paler and the silver tissue which covers the heart and the venter has become whitish.

Boophis schuboeae **Glaw & Vences, 2002**

Morphological data were assessed in one tadpole (Figures 2 and 7) in developmental stage 36 (field number FGMV 2002–1800, ZSM 978/2004, BL 12.1 mm, TL 25.5 mm, Genbank accession number DQ068394) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. schuboeae* (accession AJ315912) from the same locality. Six other voucher specimens from the same locality show the typical coloration pattern and oral disc configuration of the species.

The oral disc of the tadpoles belonging to this species is identical to those of *B. ankaratra*, except that it has a lower number of rather smaller papillae, and the lateral area where the oral disc folds is free from submarginal papillae. However, the tadpoles of these two species are easy to distinguish by their particular coloration pattern (Figure 1) (see also Glos et al. 2007). *B. schuboeae* tadpoles are characterized by the presence of up to four light and three alternating dark bands on the tail musculature. In life, the posterior part of the tail is sometimes with a contrasting orange coloration. Typically the dorsal and ventral fins originate on the tail musculature for *B. schuboeae* while they commonly originate on the body-tail junction for *B. ankaratra*.

Boophis albipunctatus **Glaw & Thiesmeier, 1993**

Morphological data were assessed in one tadpole (Figures 2 and 8) in developmental stage 25 (field number ZCMV 4946, ZSM 82/2008, BL 7.5 mm, TL 15.5 mm, Genbank accession number GU974374) from Ambohitsara-Tsitolaka. The 16S rDNA sequence of this specimen is 99% identical to a reference sequence of a *B. albipunctatus* adult specimen (accession AY848446) from Manantantely. One other voucher tadpole of *B. albipunctatus* from the same locality is morphologically very similar to the described voucher specimen.

Figure 1. Coloration in life of strongly rheophilous tadpoles of *Boophis* (dorsal, lateral and ventral views): **A** *B. andohahela* (T 09/273-ZSM 282/2009) **B** *B. ankaratra* (ZCMV 4917-ZSM 876/2007) **C** *B. schuboeae* (T 09/980-743/2008) **D** *B. sibilans* (ZCMV 11548 - to be catalogued in ZSM) **E** *B. luciae* (ZCMV 11548-to be catalogued in ZSM) **F** *B. albipunctatus* (ZCMV 4946-ZSM 82/2008) **G** *B. mandraka* [Ca38] (ZCMV 4261-ZSM 456/2007) **H** *B. sambirano* [Ca47] (ZCMV 13105-ZSM 482/2010) **I** *B. sambirano* [Ca48] (ZCMV 13109-ZSM 486/2010) **J** *B. sambirano* [Ca49] (ZCMV 13155-ZSM 528/2010) **K** *B. sambirano* [Ca50] (ZCMV 13172-ZSM 545/2010) **L** *B. marojezensis* [Ca51] (ZCMV 13550-ZSM 721/2010) **M** *B. marojezensis* [Ca52] (ZCMV 13168-ZSM 541/2010) **N** *B. marojezensis* [Ca53] (ZCMV 13200-ZSM 573/2010).

Figure 2. Pictures of the oral discs of the voucher specimens (stained with methylene blue for better visibility of morphological structures). Scale bars represent 1 mm.

B. albipunctatus tadpoles can be distinguished from those of *B. ankaratra* and *B. schuboeae* by the absence of the lateral bulge on the oral disc, the absence of the medial convexity on the upper sheath, the high number of papillae, and the LTRF 7(5–7)/3,

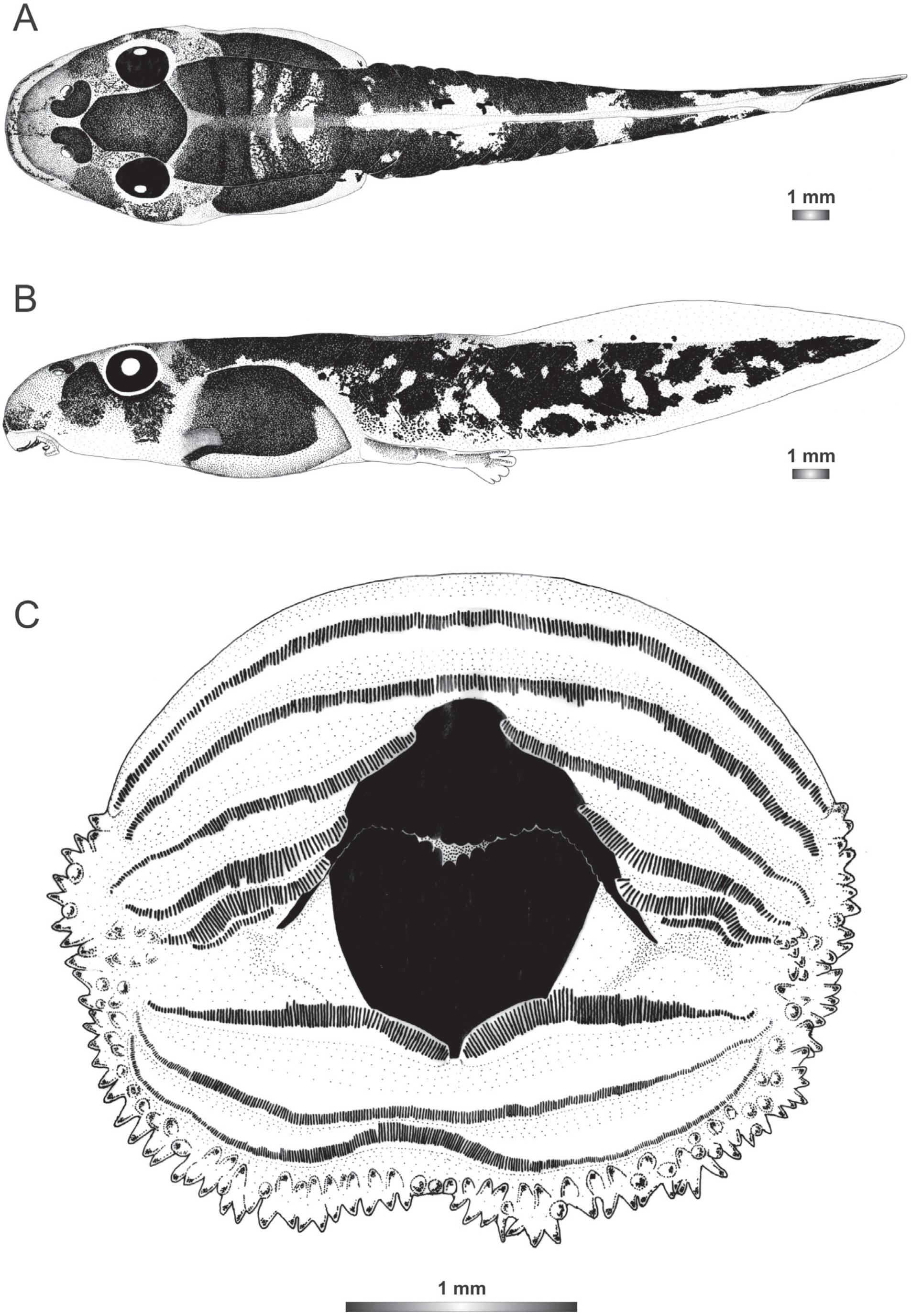

Figure 3. Drawings of the preserved DNA voucher tadpole of *Boophis englaenderi* (FGZC 2244-ZSM 623/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.

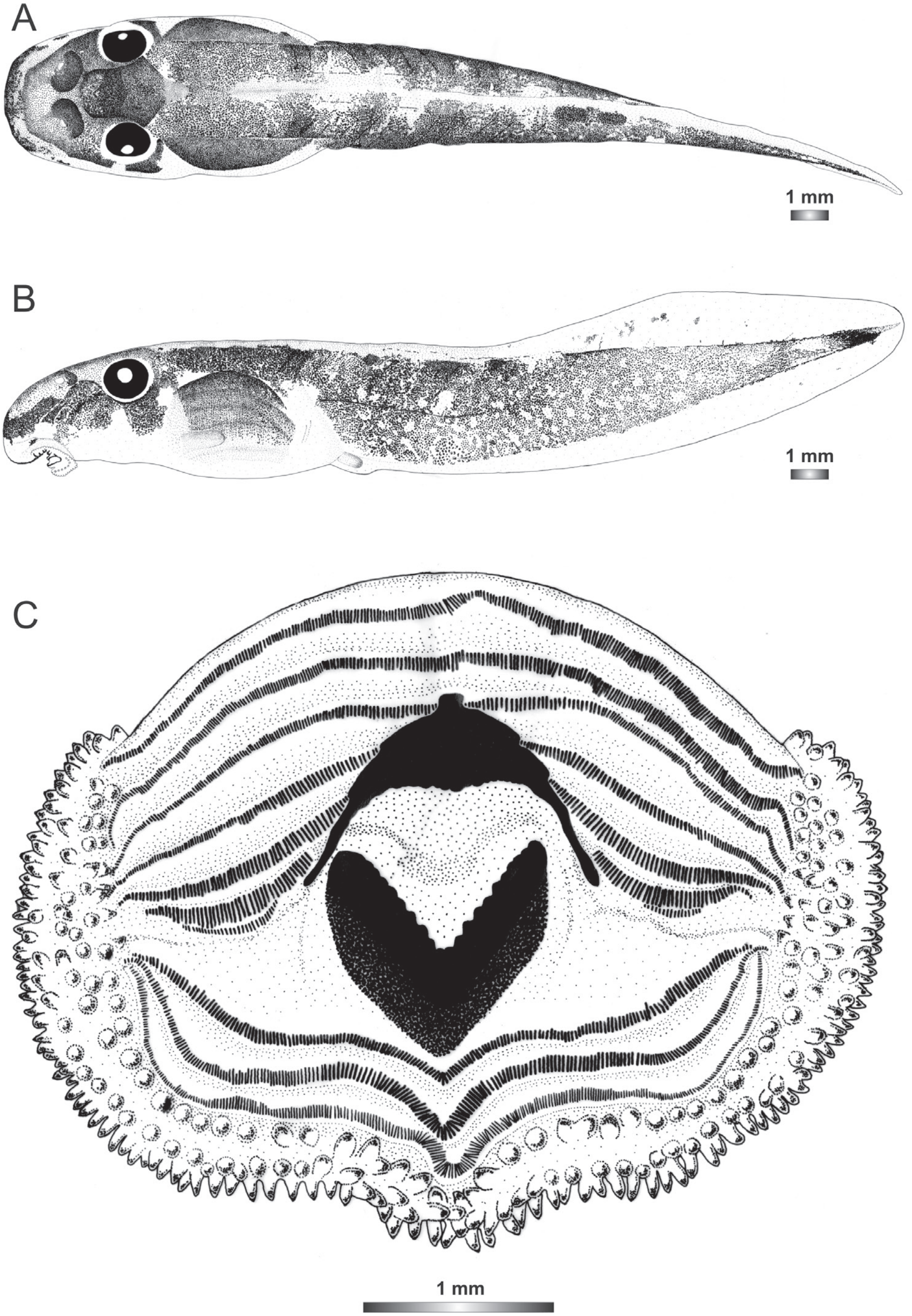

Figure 4. Drawings of the preserved DNA voucher tadpole of *Boophis englaenderi* [Ca23] (FGZC 2957-ZSM 1632/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

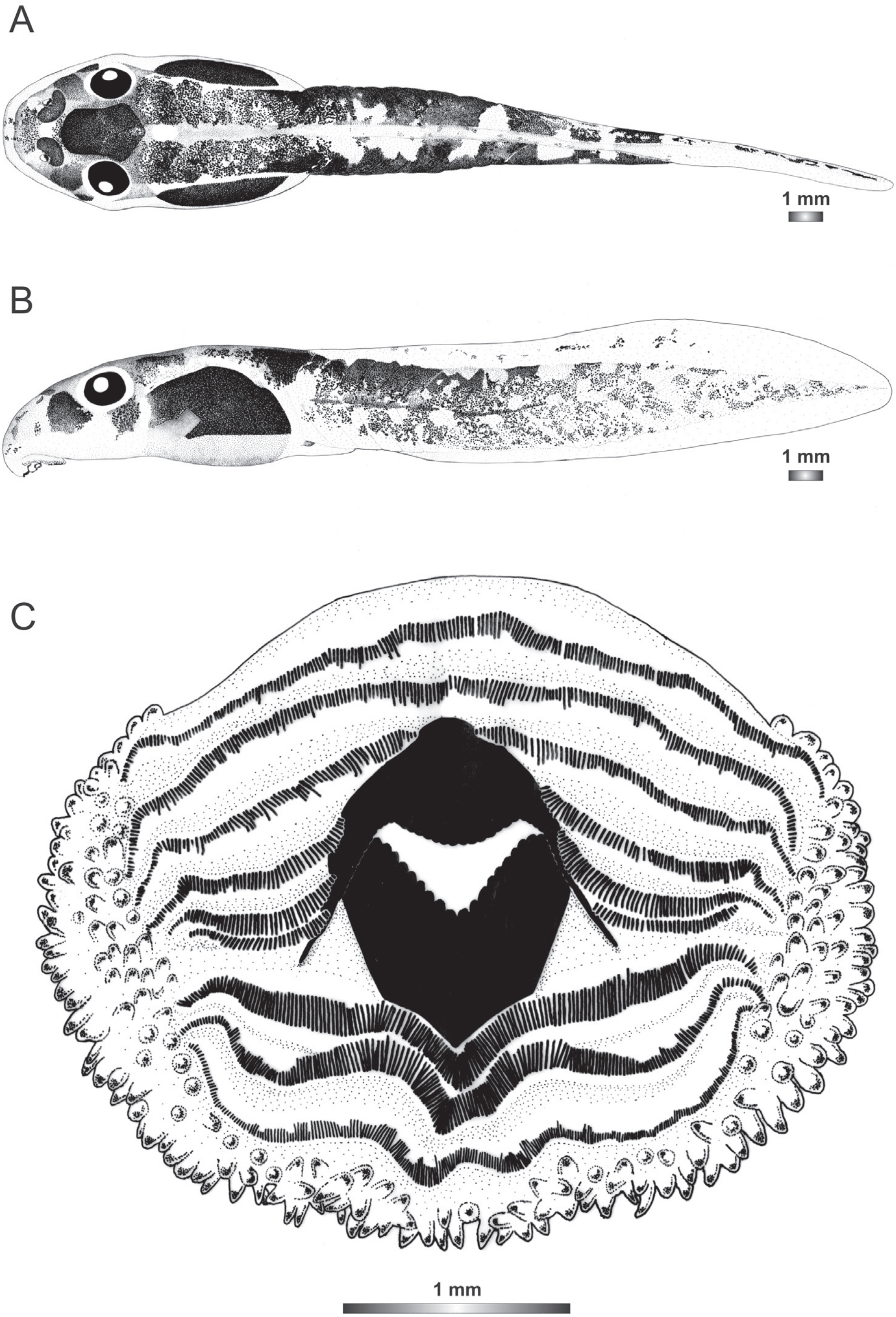

Figure 5. Drawings of the preserved DNA voucher tadpole of *Boophis andohahela* (T 428-ZSM 998/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

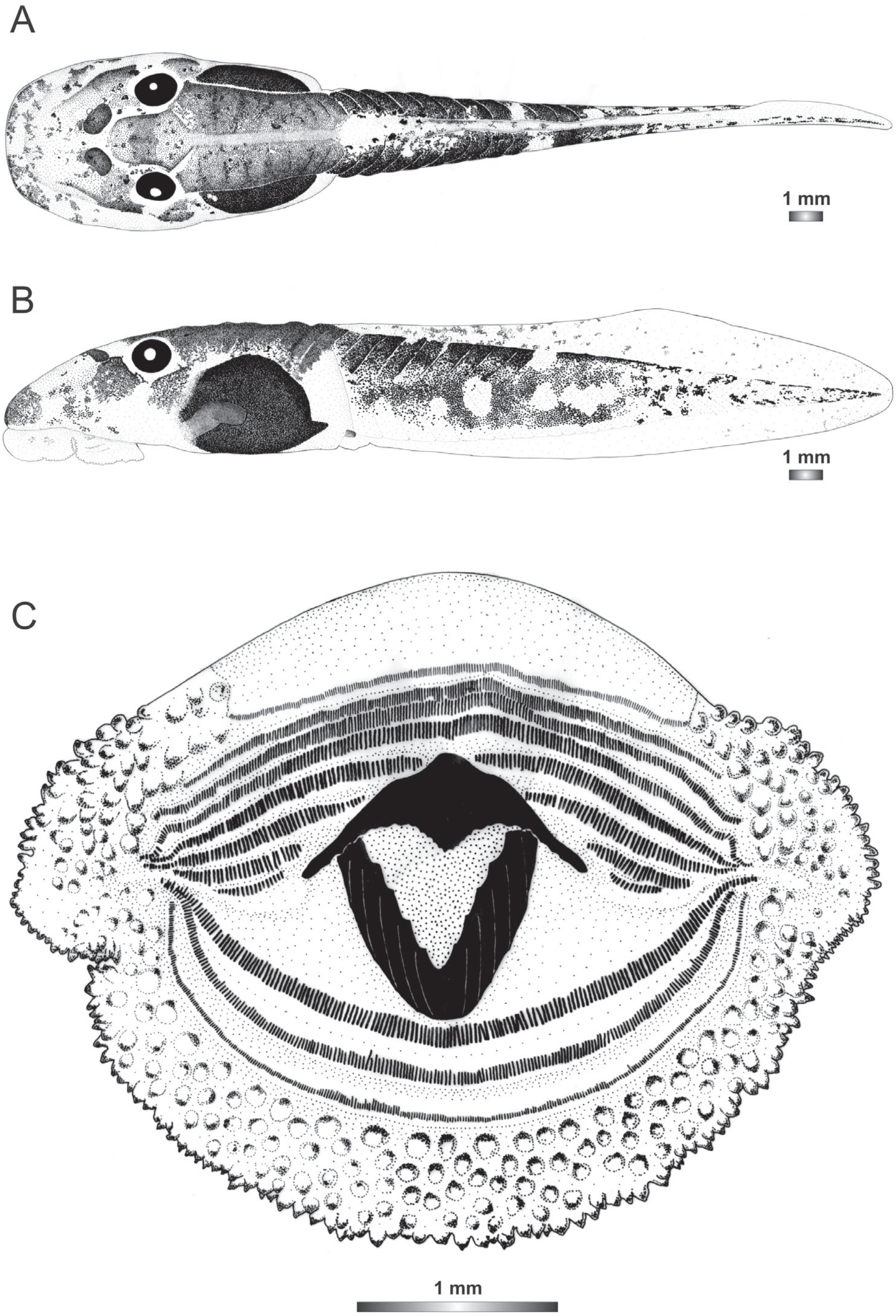

Figure 6. Drawings of the preserved DNA voucher tadpole of *Boophis ankaratra* (ZCMV 4917-ZSM 876/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

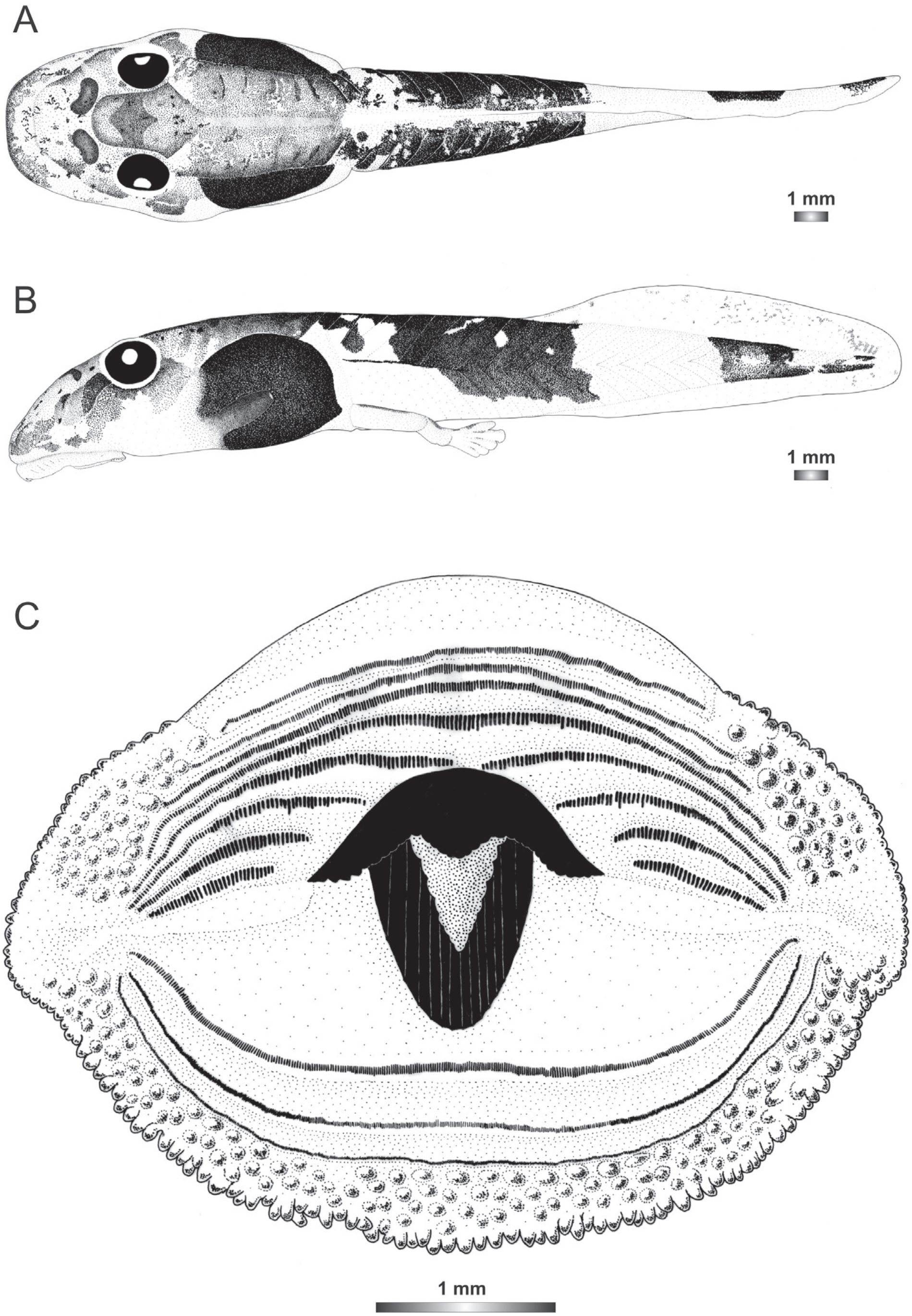

Figure 7. Drawings of the preserved DNA voucher tadpole of *Boophis schuboeae* (FG/MV 2003.1800-ZSM 978/2004): **A** Dorsal view **B** Lateral view **C** Oral disc.

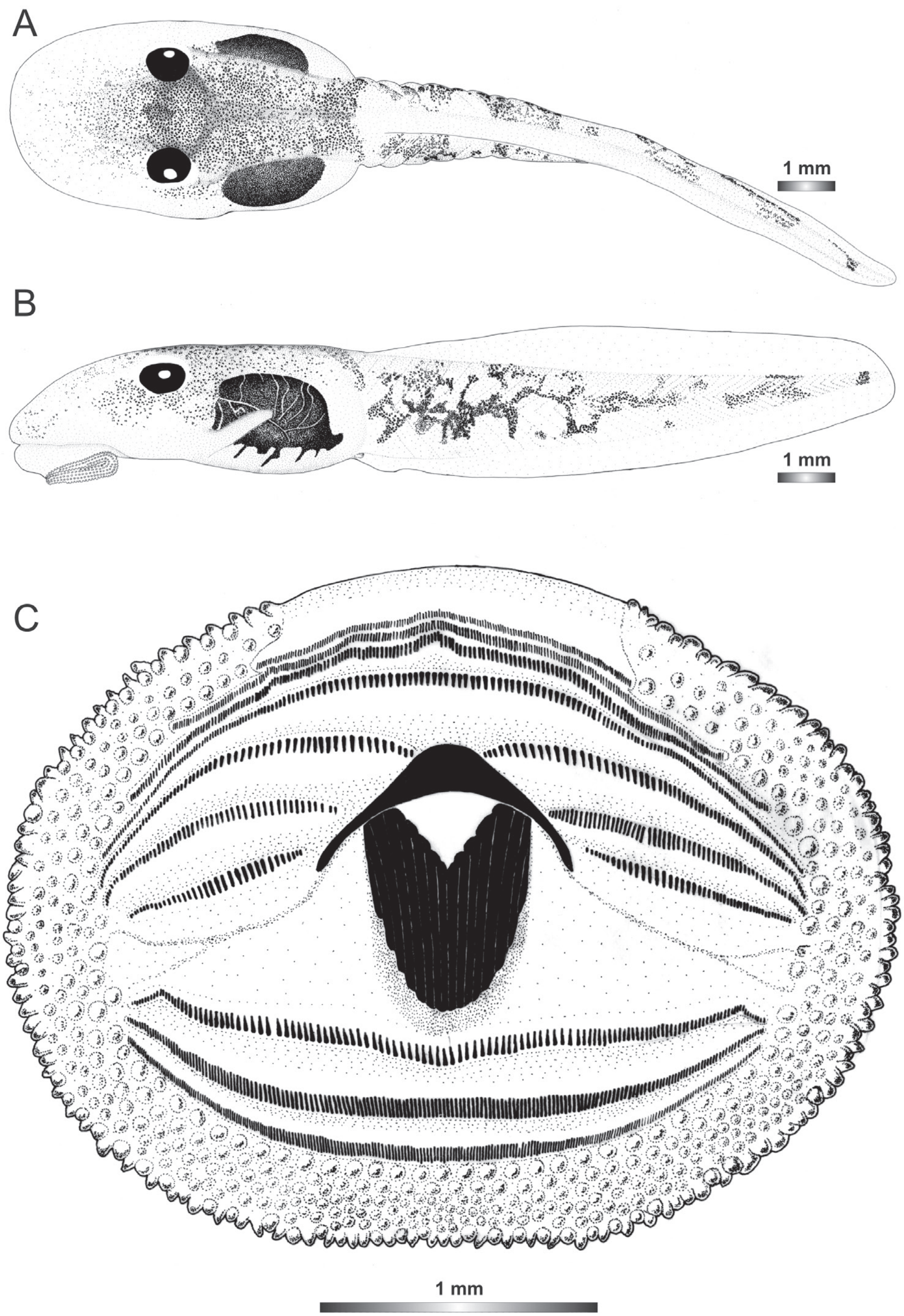

Figure 8. Drawings of the preserved DNA voucher tadpole of *Boophis albipunctatus* (ZCMV 4946-ZSM 82/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.

but they share the ribbed pattern, the U-shape, and the partially hidden state of the lower jaw sheath. These tadpole are also characterized by their less pigmented state in preservative which makes them easy to identify. The absence of silver pigment covering the heart in life is also typical for these tadpoles.

Boophis sibilans **Glaw & Thiesmeier, 1993**

Morphological data were assessed in one tadpole (Figures 2 and 9) in developmental stage 29 (field number FGZC 2956, ZSM 1631/2007, BL 11 mm, TL 26 mm, Genbank accession number JQ518194) from Marojejy National Park. The 16S rDNA sequence of this specimen is 99.4% identical to a reference sequence of a *B. sibilans* adult specimen (accession AY341718) from Andasibe. Two other voucher tadpoles have similar morphological characteristics.

B. sibilans tadpoles have the same oral disc feature (absence of lateral bulge, LTRF) as *B. albipunctatus*, except for a lower number of submarginal papillae and a V-shaped lower sheath. These tadpoles are characterized by their rather long tail (up to 200% of BL) and their unique tail pattern which is composed of dark spots separated by a clear unpigmented area. The inner part of the spots is usually free from pigment (Figure 1).

Boophis luciae **Glaw, Köhler, de la Riva, Vieites & Vences, 2010**

Morphological data were assessed in one tadpole (Figures 2 and 10) in developmental stage 36 (field number ZCMV 5146, ZSM 730/2007, BL 10.4 mm, TL 22.2 mm, Genbank accession number GU975069) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of a *B. luciae* adult specimen (accession AY848444) from the same locality. Ten other voucher tadpoles are morphologically very similar to the described voucher specimen.

The tadpoles of *B. luciae* are similar to those of *B. sibilans* by their oral disc structure and the general external pattern except that they have a rather short tail. They can be characterized by the state of the spots on the tail musculature which are connected to each other (Figure 1).

Boophis mandraka **group**

This group is characterized by tadpoles having an enlarged oral disc without lateral emargination and ventral gap of papillae. The dorsal gap of papillae is narrow to very narrow, and the lateral area where the oral disc folds is free of submarginal papillae. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the

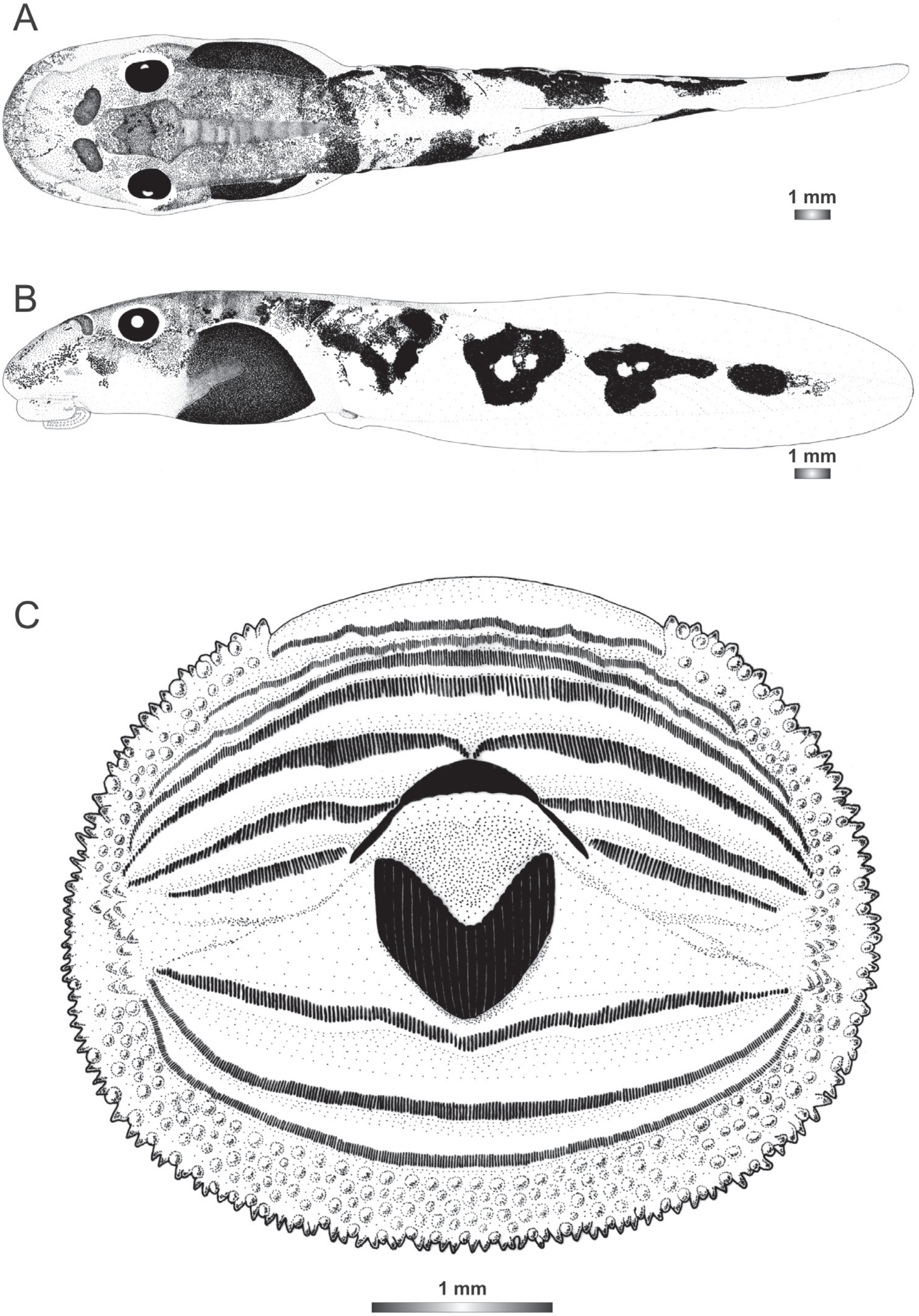

Figure 9. Drawings of the preserved DNA voucher tadpole of *Boophis sibilans* (FGZC 2956-ZSM 1631/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

entire margin is free from the snout. The upper labium has always five uninterrupted and three interrupted keratodont rows, and the three lower rows are always uninterrupted giving a unique LTRF 8(6–8)/3. The upper sheath is always absent. The lower sheaths are moderately strong and completely keratinized, U-shaped, ribed, and higher than wide. Dorsolateral glands are present.

B. sambirano Vences & Glaw, 2005

Morphological data were assessed in one tadpole (Figures 2 and 11) in developmental stage 25 (field number FG/MV 2002.1902, ZSM 672/2004, BL 6.5 mm, TL 12.7 mm, Genbank accession number EU717861) from a site locally named "Camp Norbert" in Manongarivo Special Reserve. The 16S rDNA sequence of this specimen is 96% identical to a reference sequence of *B. sambirano* adult specimen (accession AY848544), and because of this 4% difference its identity and belonging to the "true" *B. sambirano* needs further confirmation. Since this specimen was collected next to the type locality of *B. sambirano* in Manongarivo, following a parsimonious approach we here assign it to this species, although the large numbers of distinct lineages in *B. sambirano* make it likely that yet another candidate species of this complex occurs in Manongarivo. Many non-voucher specimens of the same series present morphological similarities to the voucher specimen.

B. sambirano tadpoles are easy to distinguish from all other tadpoles described above by the state of their oral disc which has no upper jaw sheath, a short keratodont row A_1, and a narrow dorsal gap of papillae. The absence of submarginal papillae on the lateral area where the oral disc folds is shared with *B. schuboeae*. The tadpoles of this species are also characterized by the extension of an obvious lateral transparent area of the body wall only on the anterior 2/3 of the body, but not surrounding the whole body like in other tadpoles.

Boophis mandraka [Ca38 Vieites et al. 2009]

Morphological data were assessed in one tadpole (Figures 2 and 12) in developmental stage 26 (field number ZCMV 4261, ZSM 456/2007, BL 7.6 mm, TL 15.8 mm, Genbank accession number FJ559153) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 93.3 % identical to a reference sequence of a *Boophis sambirano* adult specimen (accession EU717863) from Manongarivo Special Reserve.

The single tadpole of this candidate species has a similar oral disc structure to *B. sambirano* except that it has a slightly wider dorsal gap of papillae (DG/ODW 39% *vs.* 34%). The typical coloration, yellowish in life (Figure 1) and whitish in preservative and the good visibility of the 10 (5 right and 5 left) dorsolateral glands allow its distinction from other tadpoles.

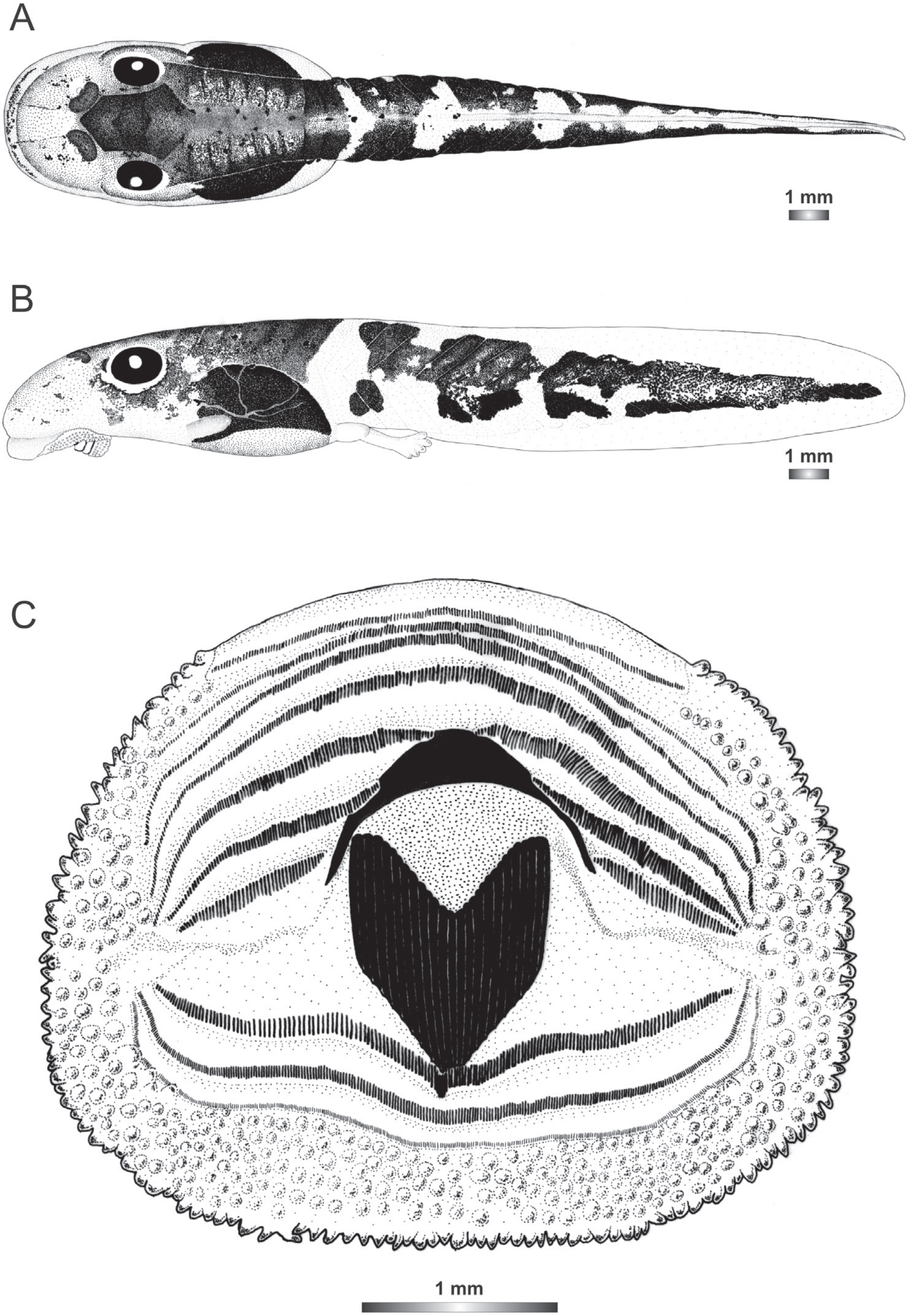

Figure 10. Drawings of the preserved DNA voucher tadpole of *Boophis luciae* (ZCMV 5146-ZSM 730/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

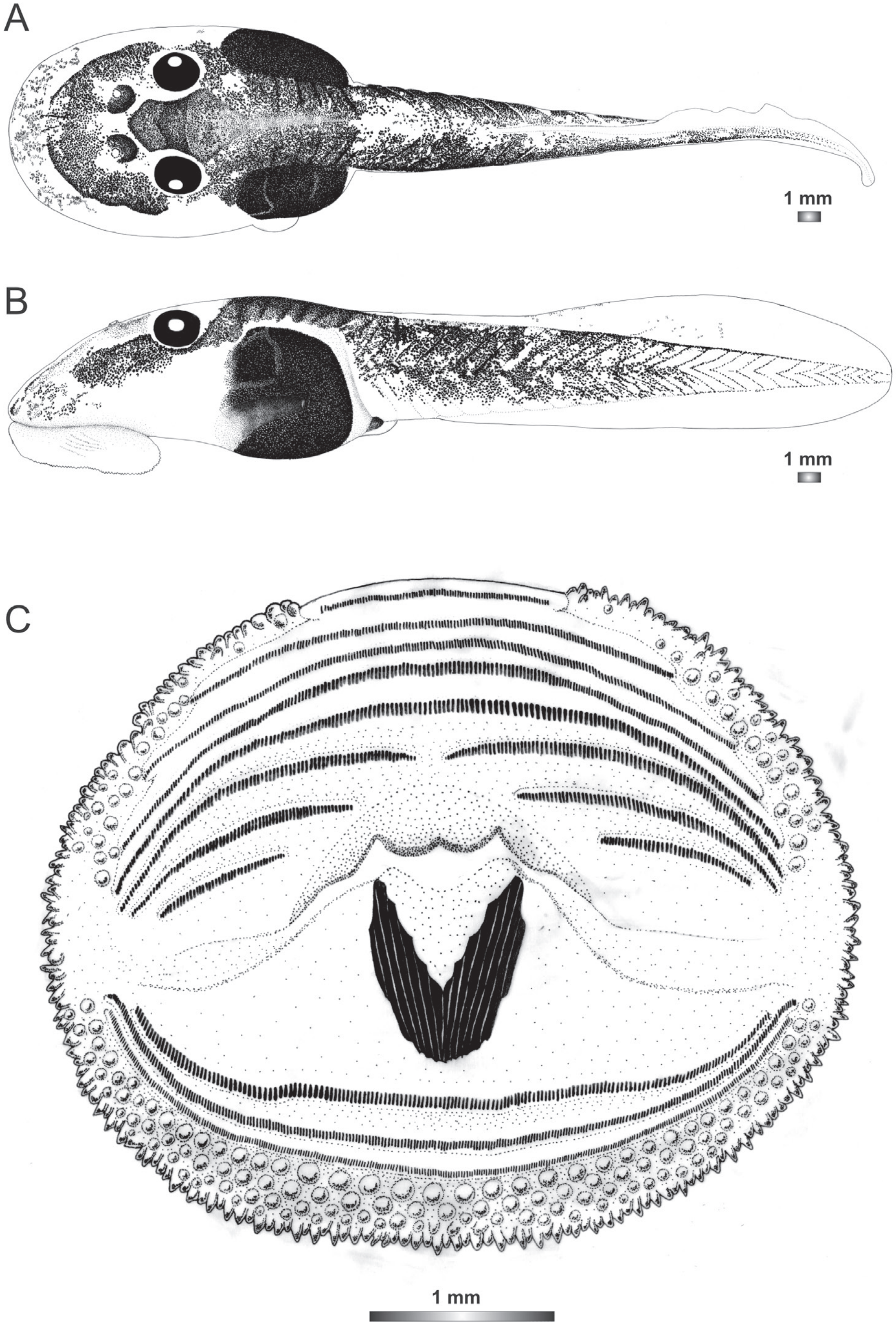

Figure 11. Drawings of the preserved DNA voucher tadpole of *Boophis sambirano* (FG/MV 2002.1904-ZSM 678/2004): **A** Dorsal view **B** Lateral view **C** Oral disc.

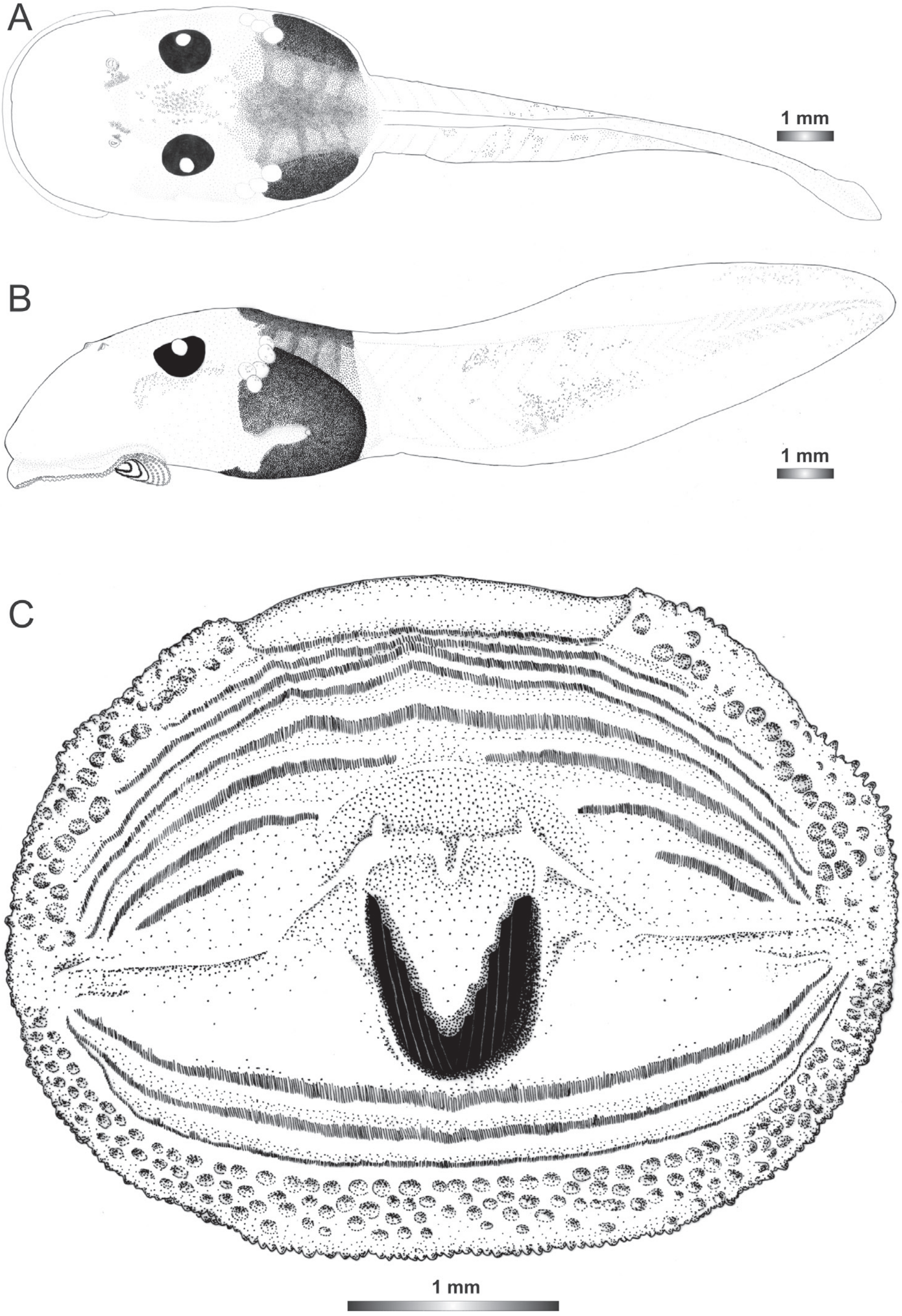

Figure 12. Drawings of the preserved DNA voucher tadpole of *B. mandraka* [Ca38] (ZCMV 4261-ZSM 456/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

Boophis mandraka [Ca46 JQ518195]

Morphological data were assessed in one tadpole (Figures 2 and 13) in developmental stage 25 (field number ZCMV 3479, ZSM 1784/2007, BL 6.8 mm, TL 14.3 mm, Genbank accession number JQ518195) from An'Ala. The 16S rDNA sequence of this specimen is 90.4 % identical to a reference sequence of *Boophis* sp. aff. *mandraka* adult specimen (accession AY848542) from Ilampy.

The oral disc of the single tadpole of this candidate species is similar to those of *B. sambirano* and *B. mandraka* [Ca38] except that it has the narrowest dorsal gap of papillae with DG 14% of ODW and the shortest A_1 with 21% of ODW. Within the *Boophis mandraka* group tadpoles, it has also the lowest number of papillae. The external morphology of the single tadpole of this candidate species is similar to that of tadpoles of *B. sambirano*, except that the ratio RN/NP is much higher (194 *vs.* 125) and the pigmentation pattern is slightly different.

Boophis sambirano [Ca47 JQ518203]

Morphological data were assessed in one tadpole (Figures 2 and 14) in developmental stage 27 (field number ZCMV 13105, ZSM 482/2010, BL 13.5 mm, TL 27.1 mm, Genbank accession number JQ518203) from Anjingo river (bridge 57 km from Antsohihy to Bealanana). The 16S rDNA sequence of this specimen is 97% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Two other voucher tadpoles are morphologically very similar to the described voucher specimen.

The tadpoles assigned to this candidate species have a similar oral disc structure as *B. sambirano* exept that they have a higher number of marginal papillae (377 *vs.* 248) and of keratodonts on A_3 (1193 *vs.* 740). These tadpoles have a rather large size in comparison to others of the *B. mandraka* group, and their pigmentation pattern distinguishes them also. Their tail musculature is covered by dissipated distinct patches following mainly the lateral tail vein and the myosepta on the anterior half of the tail musculature, and irregularly dispersed on the posterior half (Figure 1), whereas it is just covered by dense spots on the anterior half in *B. sambirano* tadpoles. The dorsal fin of these tadpoles begins usually on the anterior 1/5 of the tail musculature, *vs.* beginning more or less at the dorsal body-tail junction in *B. sambirano*.

Boophis sambirano [Ca48 JQ518205]

Morphological data were assessed in one tadpole (Figures 2 and 15) in developmental stage 27 (field number ZCMV 13109, ZSM 485/2010, BL 12.7 mm, TL 24.7

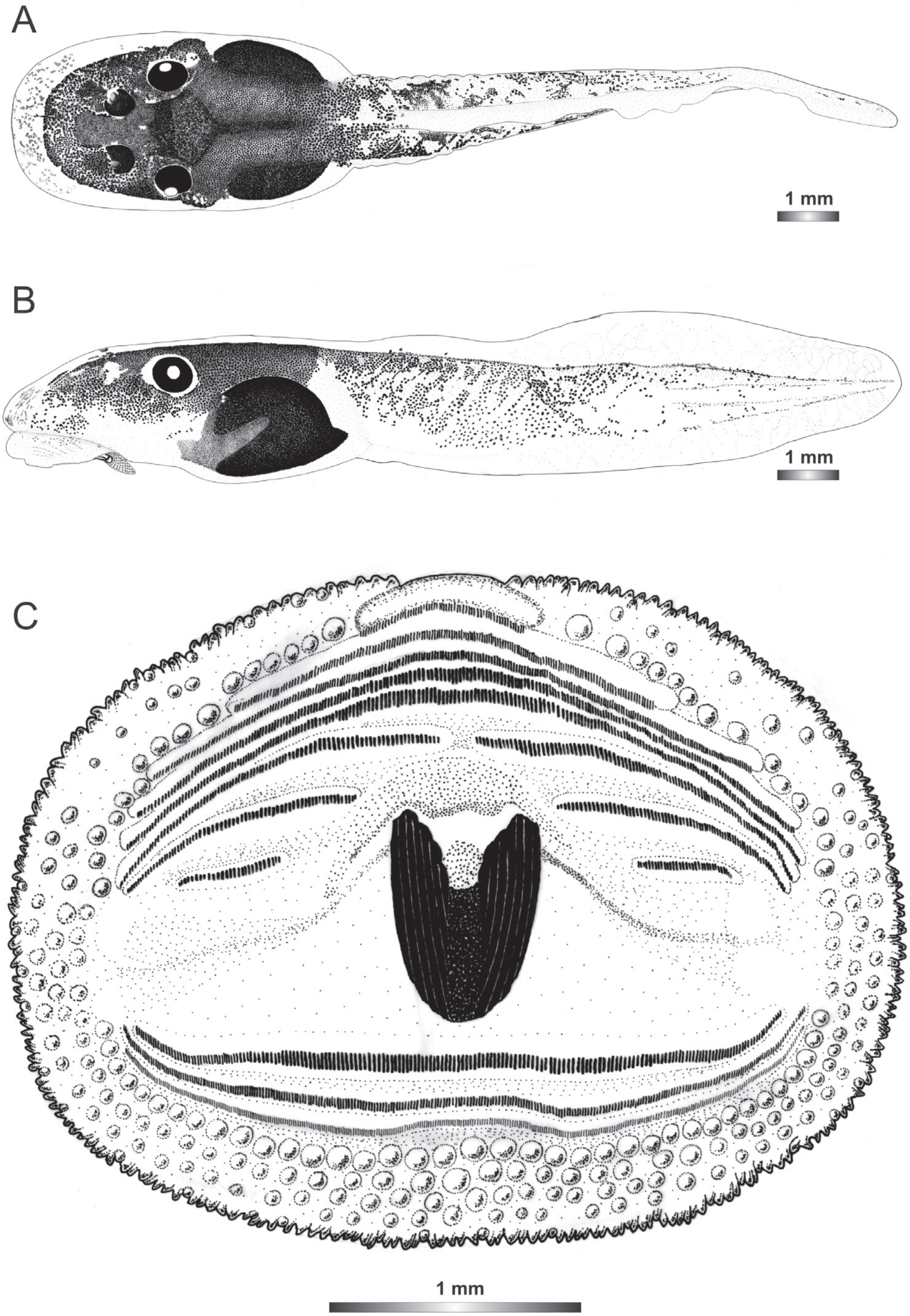

Figure 13. Drawings of the preserved DNA voucher tadpole of *B. mandraka* [Ca46] (ZCMV 3479-ZSM 1784/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

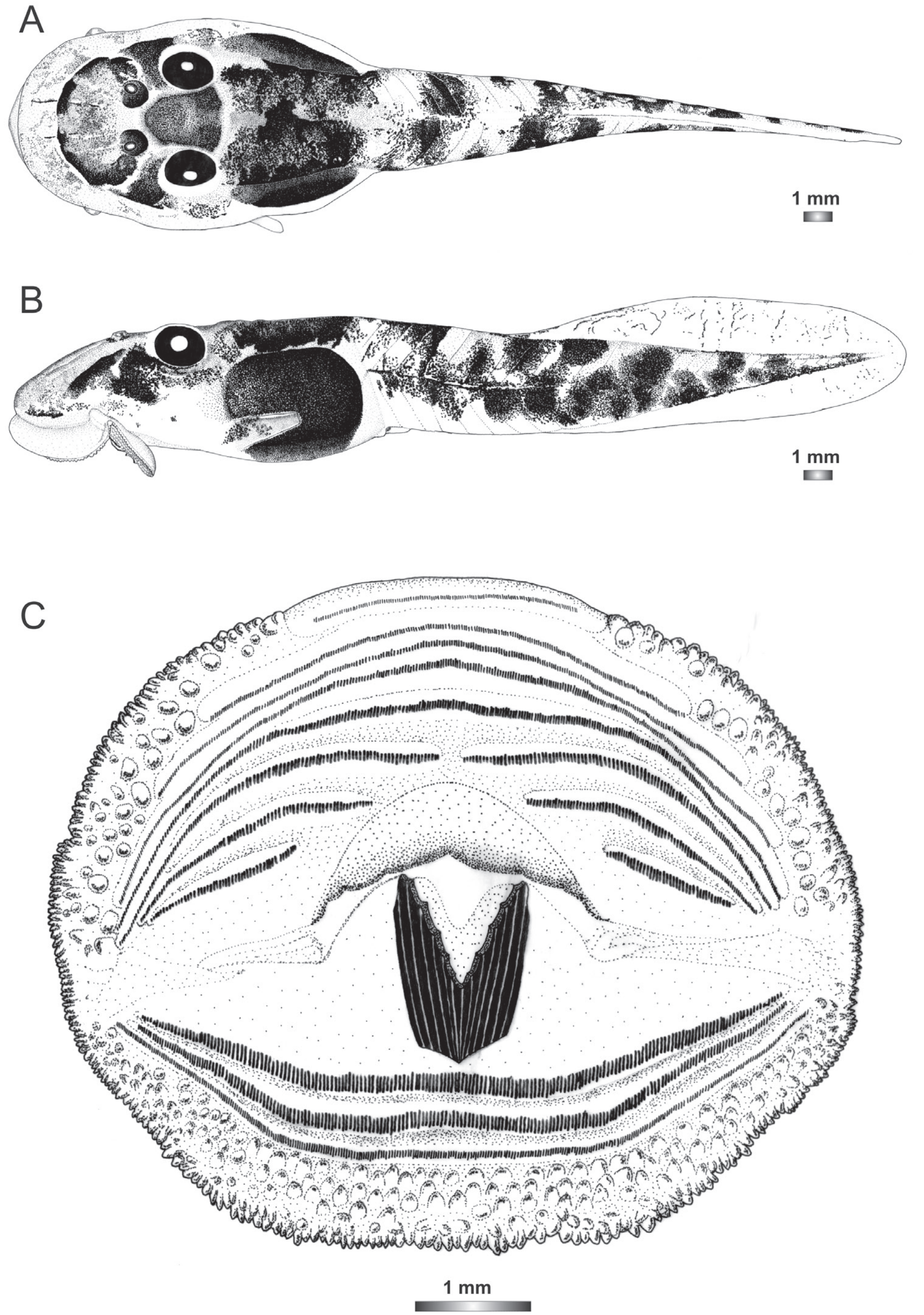

Figure 14. Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca47] (ZCMV 13105-ZSM 482/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

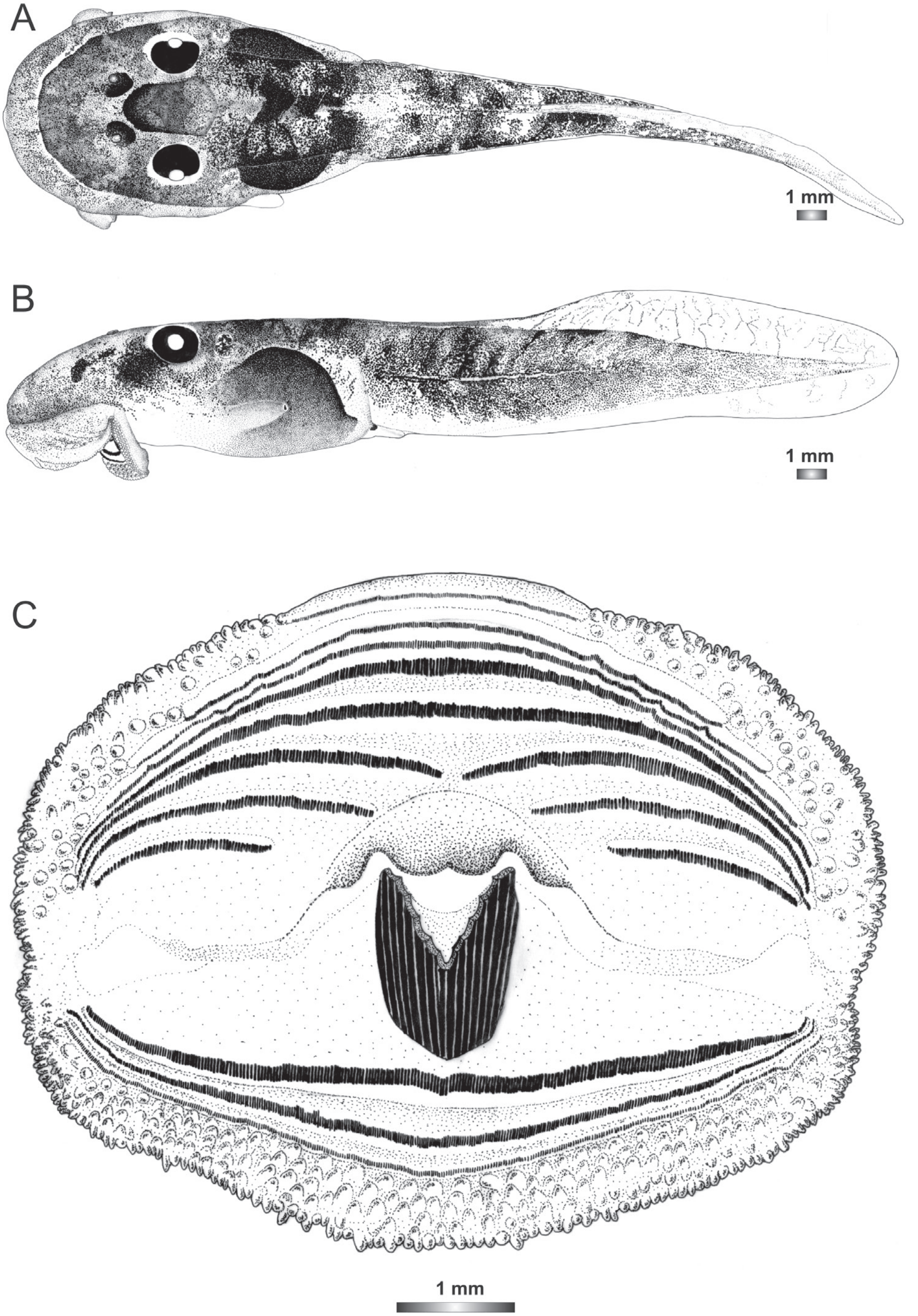

Figure 15. Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca48] (ZCMV 13109-ZSM 485/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

mm, Genbank accession number JQ518205) from Anjingo river (bridge at 57 km on the road from Antsohihy to Bealanana). The 16S rDNA sequence of this specimen was 94% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Two other voucher tadpoles are very similar to the described voucher specimen.

The tadpoles assigned to this candidate species have a similar oral disc as *B. sambirano* and *B. sambirano* [Ca47]. The higher number of marginal papillae (336) and of keratodonts on A_3 (1052) differentiate these tadpoles from those of *B. sambirano* but are similar to *B. sambirano* [Ca47]. The ovoidal body form in dorsal view and the pigmentation pattern – variegated spots on the body and less coalesced spots on the tail musculature (Figure 1) – differentiate these tadpoles from those of *B. sambirano* [Ca47]. The beginning of the dorsal fin on the anterior 1/5 of the tail musculature is similar to that of *B. sambirano* [Ca47] but different from *B. sambirano*.

Boophis sambirano [Ca49 JQ518208]

Morphological data were assessed in one tadpole (Figures 2 and 16) in developmental stage 27 (field number ZCMV 13155, ZSM 528/2010, BL 11.7 mm, TL 26.7 mm, Genbank accession number JQ518208) from Ankijagna Lagnana. The 16S rDNA sequence of this specimen is 94.1% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Three other voucher specimens and many non-voucher specimens of the same series are morphologically very similar to the described specimen.

The oral disc of the tadpoles assigned to this candidate species is the typical one of the *B. mandraka* group, characterized by a narrow dorsal gap of papillae (DG 23% of ODW) which is here wider than in *B. mandraka* [Ca46] but smaller than in the other tadpoles, and the short keratodont row A_1 which is similar to that of *B. mandraka* [Ca46] tadpoles. The number of papillae is similar to that of *B. sambirano* and *Boophis mandraka* [Ca38]. These tadpoles can be easily distinguished from all *B. sambirano*-like tadpoles by their particular pigmentation pattern which is uniformly dark (Figure 1), by the non visibility of the lateral transparent area of the body wall, the ovoidal form of the body in dorsal view, and the eye position between the anterior 3/10 and 4/10 of the body.

Boophis sambirano [Ca50 JQ518211]

Morphological data were assessed in one tadpole (Figures 2 and 17) in developmental stage 27 (field number ZCMV 13172, ZSM 545/2010, BL 11.7 mm, TL 25.7 mm, Genbank accession number JQ518211) from Ambinanitelo. The 16S rDNA sequence of this specimen is 94.9% identical to a reference sequence of *Boophis sambirano* tad-

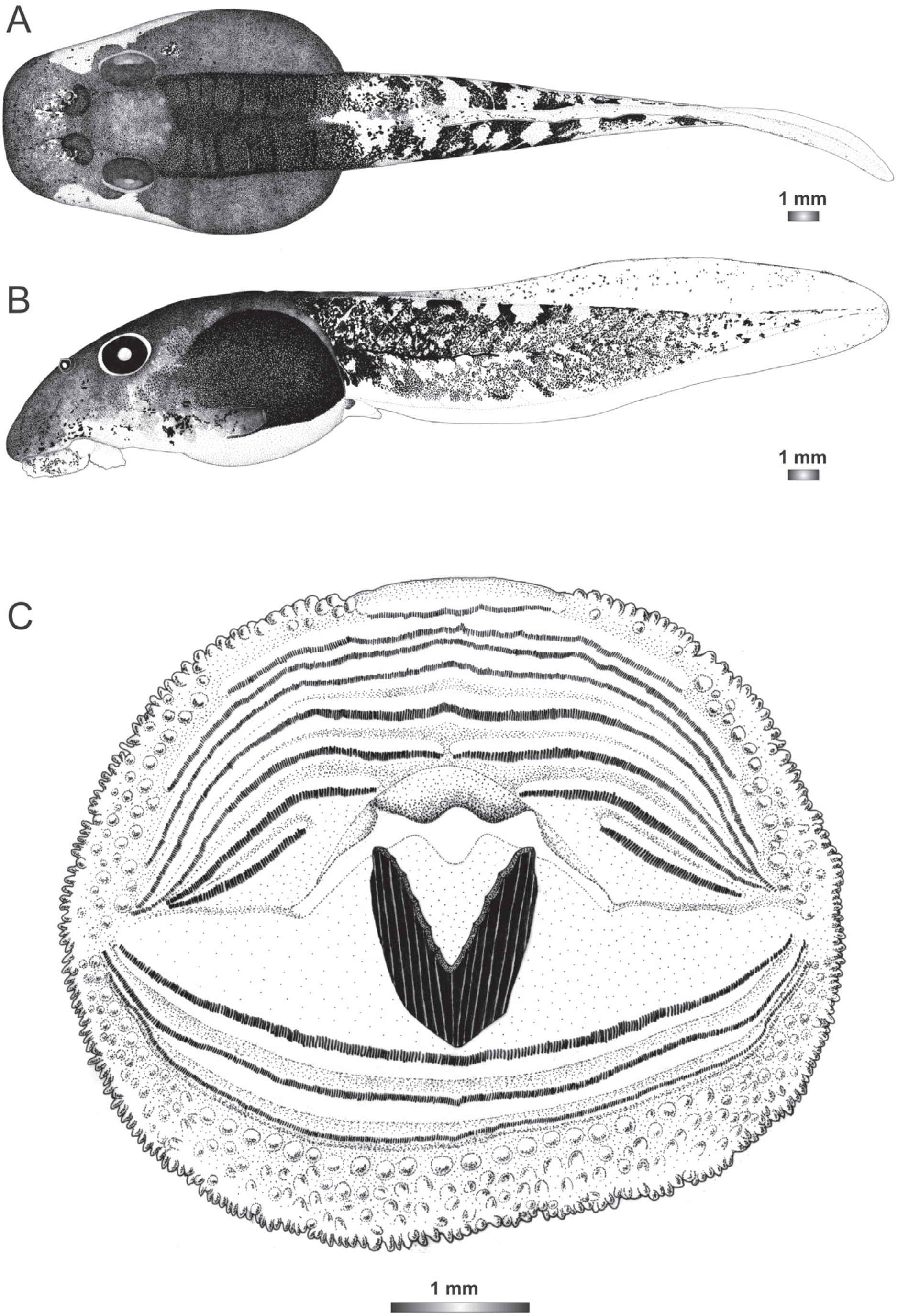

Figure 16. Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca49] (ZCMV 13155-ZSM 528/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

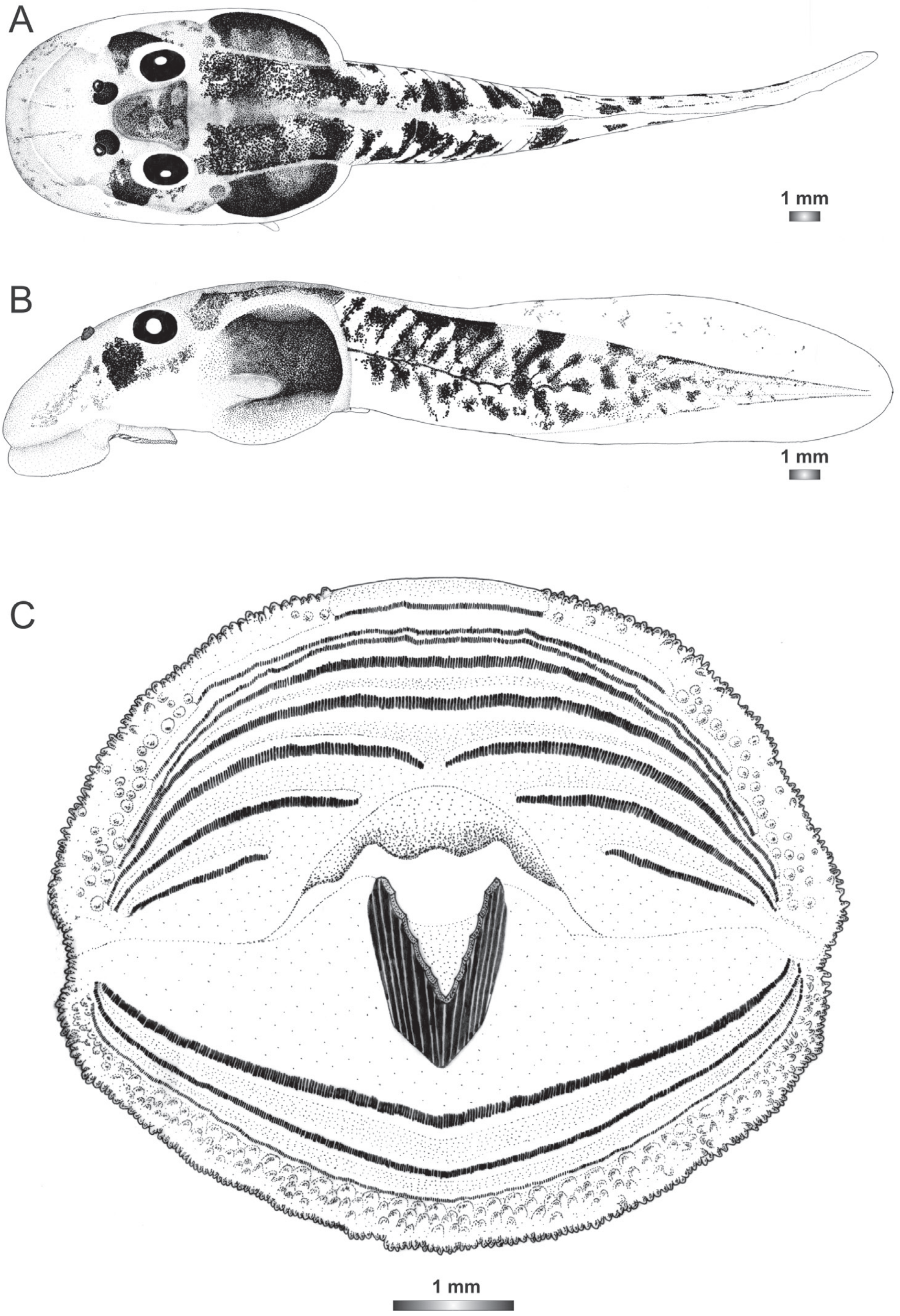

Figure 17. Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca50] (ZCMV 13172-ZSM 545/2010): **A** Dorsal view **B** Lateral view; **C** Oral disc.

poles (accession EU717861) from Manongarivo Special Reserve. Three other voucher tadpoles are morphologically very similar to the described voucher specimen.

The oral disc of the tadpoles of this candidate species is similar to that of other *B. mandraka* group species. The tadpoles belonging to this candidate species have an elliptical body form in dorsal view but differ from those of *B. sambirano* [Ca49] by their pigmentation pattern. The presence of a lateral transparent area of the body wall surrounding the anterior 2/3 of the body is similar to those of *B. sambirano*, but the absence of contrasted integumental patches limiting the transparent body wall area surrounding the snout is a difference to *B. sambirano*, *Boophis sambirano* [Ca47], and *Boophis sambirano* [Ca48]. The tadpoles of this candidate species can thus be distinguished from those of other candidate species close to *B. sambirano* mainly by their coloration pattern (Figure 1).

Boophis majori group

This group is heterogeneous in larval morphology and probably non monophyletic (e.g., Schmidt et al. 2008; Randrianiaina et al. 2009a). The rheophilous tadpoles in this group with an enlarged oral disc are further characterized by the absence of a lateral emargination, and absence of dorsal and ventral gaps of papillae. The submarginal papillae are complete. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the entire margin is free from the snout. LTRF 7(5–7)/3. The jaw sheaths are moderately strong and completely keratinized. The upper sheath always lacks a medial convexity. The lower sheath is U-shaped, ribbed, and higher than wide. Dorsolateral glands are present.

Boophis marojezensis Glaw & Vences, 1994

Morphological data were assessed in one tadpole (Figures 2 and 18) in developmental stage 27 (field number FGZC 2277, ZSM 1528/2007, BL 7.1 mm, TL 18.3 mm, Genbank accession number JQ518196), from Marojejy National Park. The 16S rDNA sequence of this specimen is 99.8% identical to a reference sequence of a *B. marojezensis* adult specimen (accession FJ559127) from the same locality. Three other voucher tadpoles are morphologically very similar to the described voucher specimen.

The tadpoles of this species are easily to distinguish from those belonging to other species groups (as described above) by the general structure of their oral disc which has no dorsal gap of papillae, and a LTRF of 7(5–7)/3. These tadpoles are also characterized by the highest number of submarginal papillae in *Boophis*, with 290 marginal and 606 submarginal papillae. The lateral transparent area of the body wall area is visible and the dorsolateral gland is obvious. The tail muscle is spotted and the spots fused

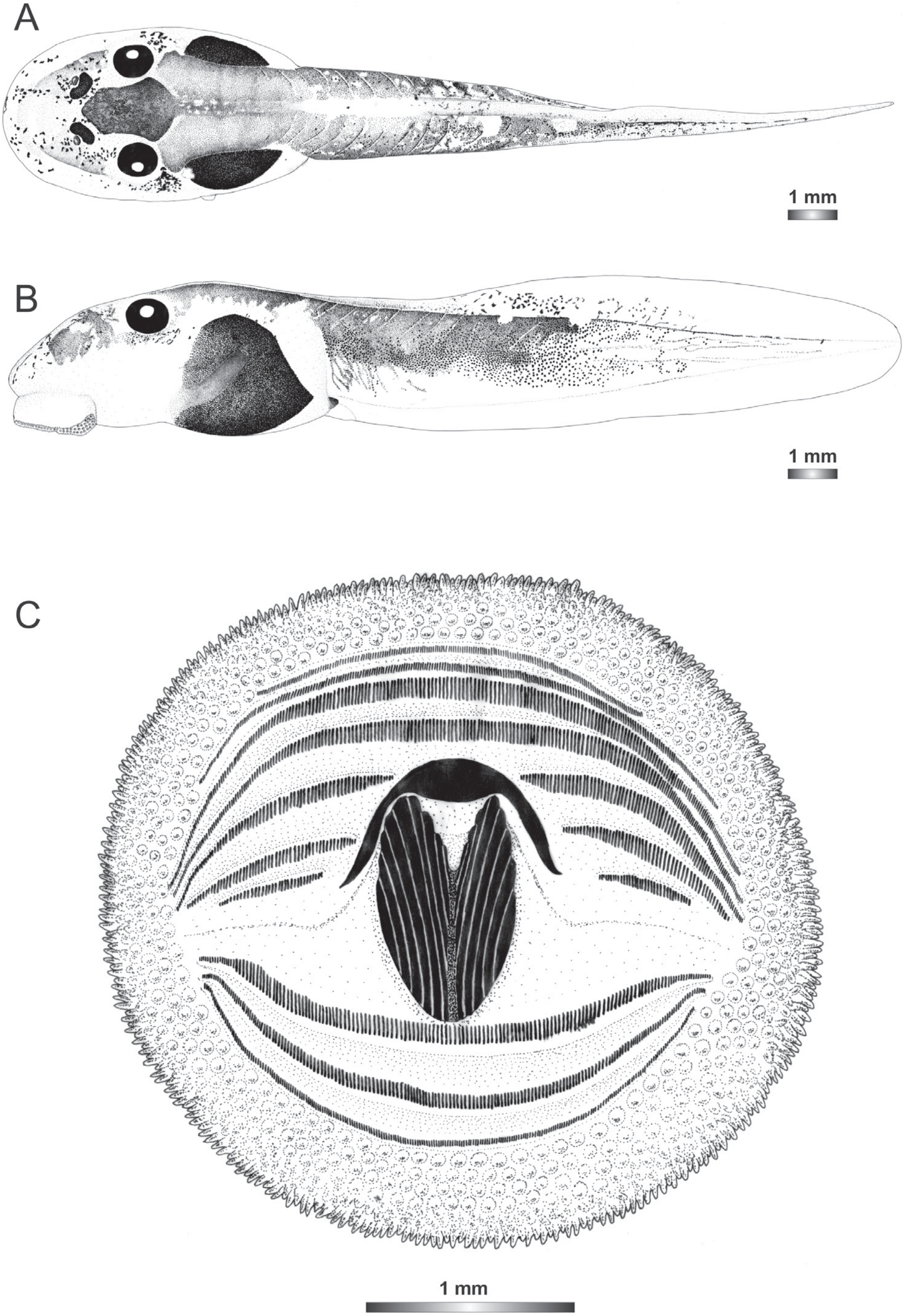

Figure 18. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* (FGZC 2277-ZSM 1528/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

to form patches mainly on the upper half of tail musculature, the density of the spots diminishes toward the tail tip. The posterior 1/3 of the tail has few pigments.

Boophis marojezensis **[Ca25 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 19) in developmental stage 29 (field number FGZC 2929, ZSM 1611/2007, BL 7.8 mm, TL 18.5 mm, Genbank accession number FJ559146), from Marojejy National Park. The 16S rDNA sequence of this specimen is 97% identical to a reference sequence of *B. marojezensis* adult specimen (accession AY848596) from Vohidrazana, and less similar to other tadpoles from Marojejy. Two non-voucher specimens from the same series have the particular caudal pattern present in the voucher specimen.

Tadpoles assigned to this candidate species have the same oral disc structure as those of *B. marojezensis*, but with a lower number of papillae (222 marginal and 315 submarginal). The presence of seven more or less rounded patches formed by condensation of spots on the posterior half of the tail musculature of these tadpoles is a further useful character to differentiate them from those of *B. marojezensis*.

Boophis marojezensis **[Ca26 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 20) in developmental stage 29 (field number FGZC 2930, ZSM 1612/2007, BL 8.8 mm, TL 20.6 mm, Genbank accession number JQ518197), from Marojejy National Park. The 16S rDNA sequence of this specimen is 96.6% identical to a reference sequence of a *B. marojezensis* adult specimen (accession AY848595) from Tsaratanana.

The single tadpole belonging to this candidate species has the typical *marojezensis*-like oral disc structure with 234 marginal and 430 submarginal papillae. It has almost the same pigmentation pattern as *B. marojezensis*, but the patches are more striking on the upper limit of tail musculature. It is differentiated from *B. marojezensis* [Ca25] by the absence of distinct patches on the tail musculature.

Boophis marojezensis **[Ca51 JQ518198]**

Morphological data were assessed in one tadpole (Figures 2 and 21) in developmental stage 25 (field number ZCMV 3691, ZSM 267/2008, BL 6 mm, TL 20 mm, Genbank accession number JQ518198) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 99.7% identical to a reference sequence of a *B. marojezensis* adult specimen (accession AY848594) from Vohiparara (but with >5% divergence

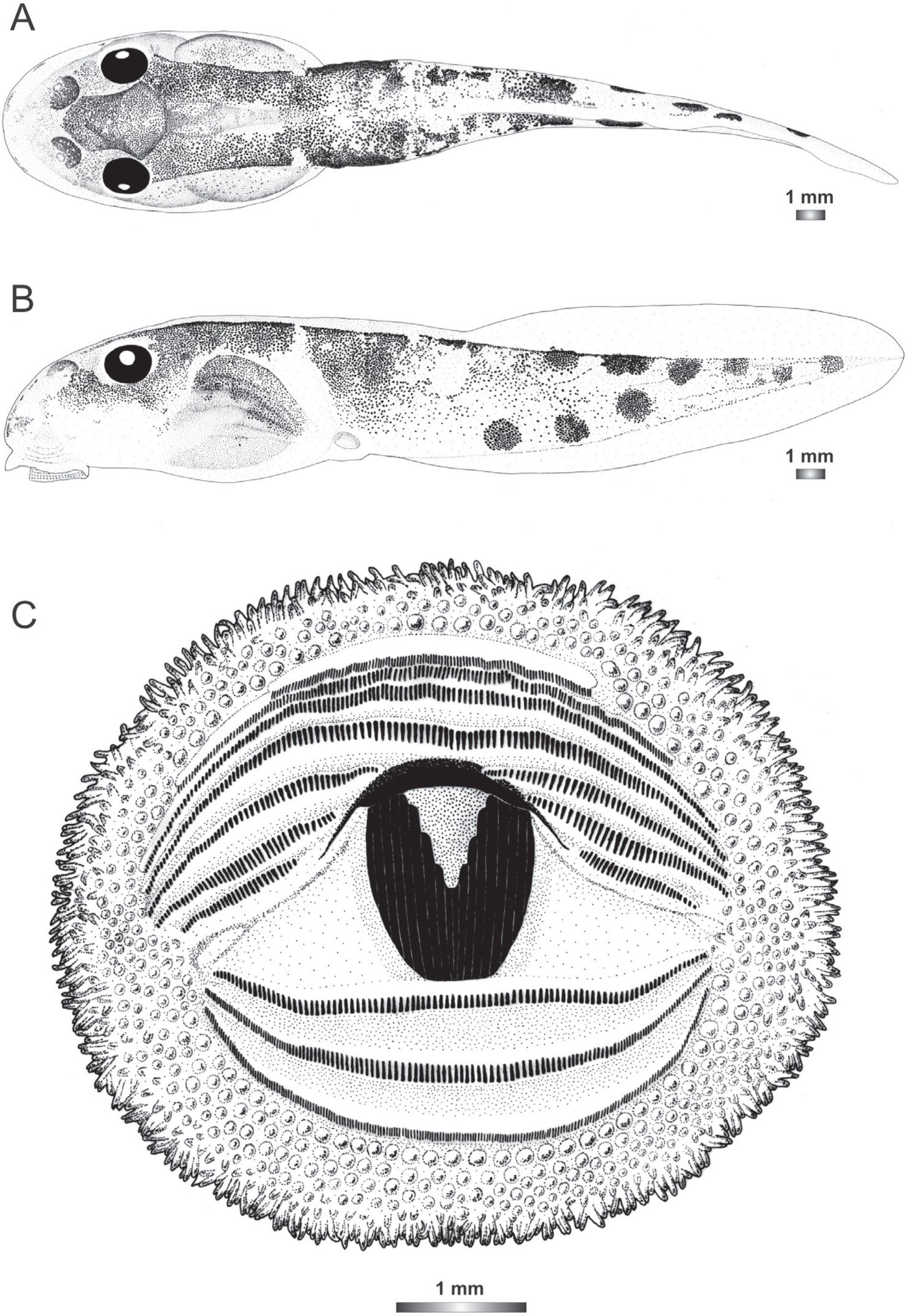

Figure 19. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca25] (FGZC 2929-ZSM 1611/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

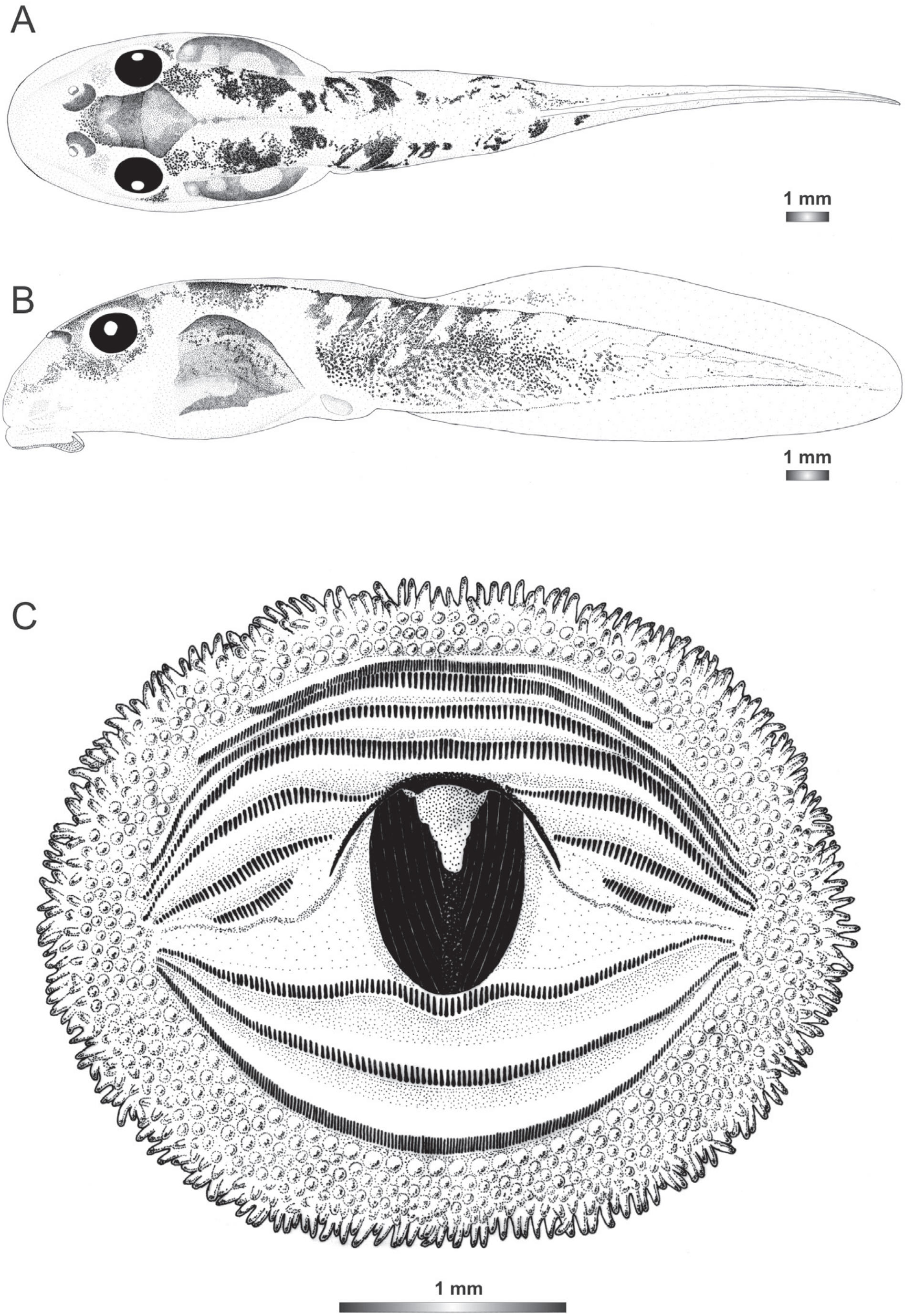

Figure 20. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca26] (FGZC 2930-ZSM 1612/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

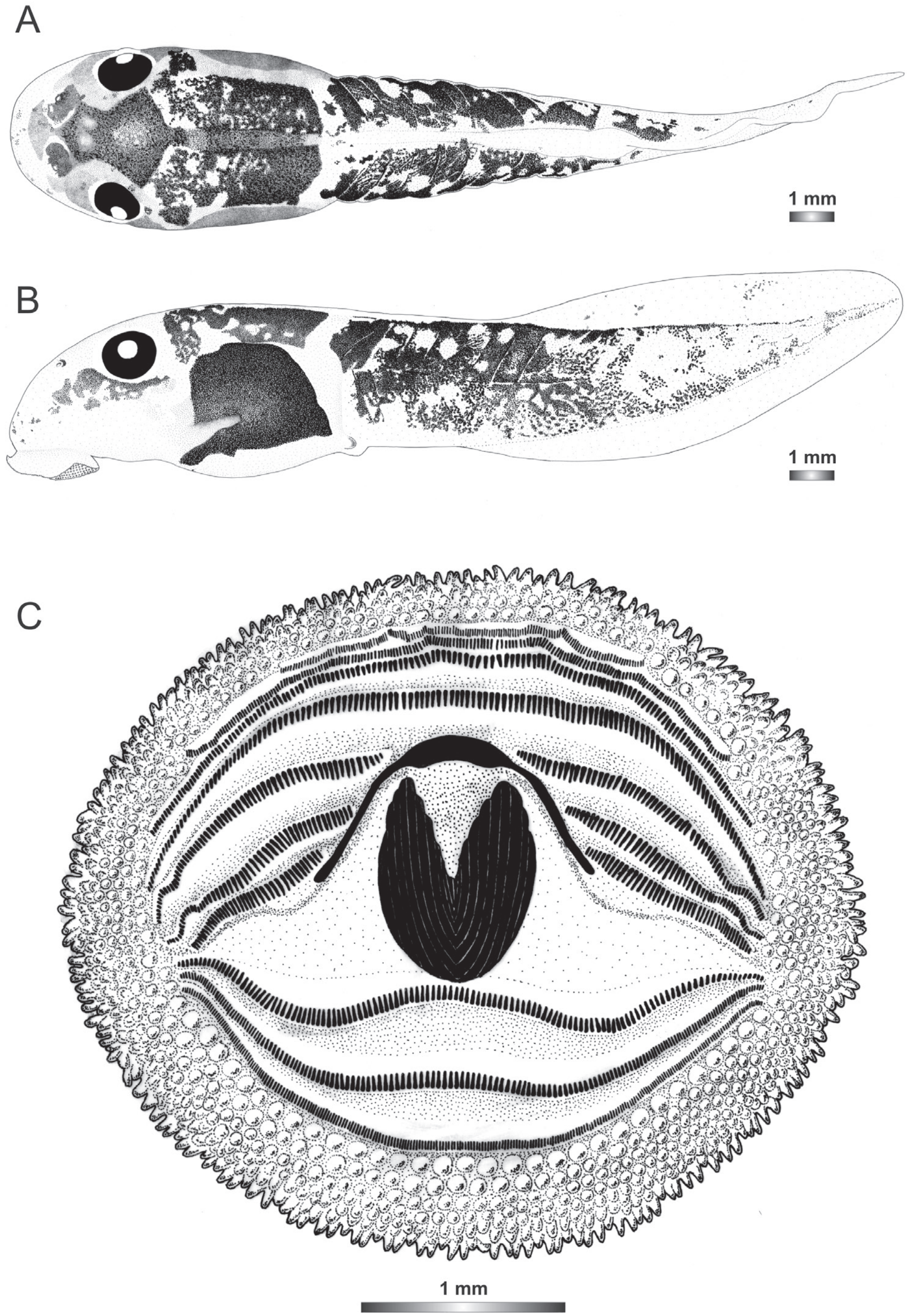

Figure 21. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca51] (ZCMV 3691-ZSM 267/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.

to all other *B. marojezensis*-like forms). Twenty-one other tadpoles assigned to this candidate species reveal a similar morphological pattern and oral disc configuration as the described voucher specimen.

The tadpoles assigned to this candidate species have the typical *marojezensis*-like oral disc structure with 297 marginal and 309 submarginal papillae. They can be distinguished from the other *marojezensis*-like tadpoles by the absence of a lateral transparent area of the body wall area surrounding the body. They have also the widest inter-orbital distance (IOD) in the group, and they are also the only *marojezensis*-like tadpoles with eyes situated between the anterior 3/10 and 4/10 of the body. The tail muscle is covered by reticulations, mainly on the anterior half.

Boophis marojezensis **[Ca52 JQ518215]**

Morphological data were assessed in one tadpole (Figures 2 and 22) in developmental stage 28 (field number ZCMV 13168, ZSM 541/2010, BL 10.5 mm, TL 26.1 mm, Genbank accession number JQ518215) from Ambinanitelo. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult specimen assigned to *B. marojezensis* (accession AY848595) from Tsaratanana (but with >5% divergence to all other *B. marojezensis*-like forms). One other voucher specimen is morphologically very similar to the described one.

Tadpoles of this candidate species have the typical *marojezensis*-like oral disc structure with 258 marginal and 522 submarginal papillae. These tadpoles are distinguished from other *marojezensis*-like tadpoles by the only poorly recognizable lateral transparent body wall area surrounding the body, and by their tail pigmentation pattern which lacks melanophoric pigments (Figure 1). The position of the eyes is in the range of most other *B. marojezensis*-like tadpoles.

Boophis marojezensis **[Ca53 JQ518216]**

Morphological data were assessed in one tadpole (Figures 2 and 23) in developmental stage 27 (field number ZCMV 13200, ZSM 573/2010, BL 9.6 mm, TL 23 mm, Genbank accession number JQ518216) from Tsaratanana Integral Reserve. The 16S rDNA sequence of this specimen is 98.8% identical to a reference sequence of a *B. marojezensis* adult specimen (accession FJ559127) from Marojejy. Five other voucher specimens attributed to the same candidate species are morphologically very similar to the described one.

The tadpoles of this candidate species have also a *marojezensis*-like oral disc with 243 marginal and 452 submarginal papillae. They are similar to *B. marojezensis*, *B. marojezensis* [Ca25], and *B. marojezensis* [Ca26], but different from *B. marojezensis* [Ca51] and *B.*

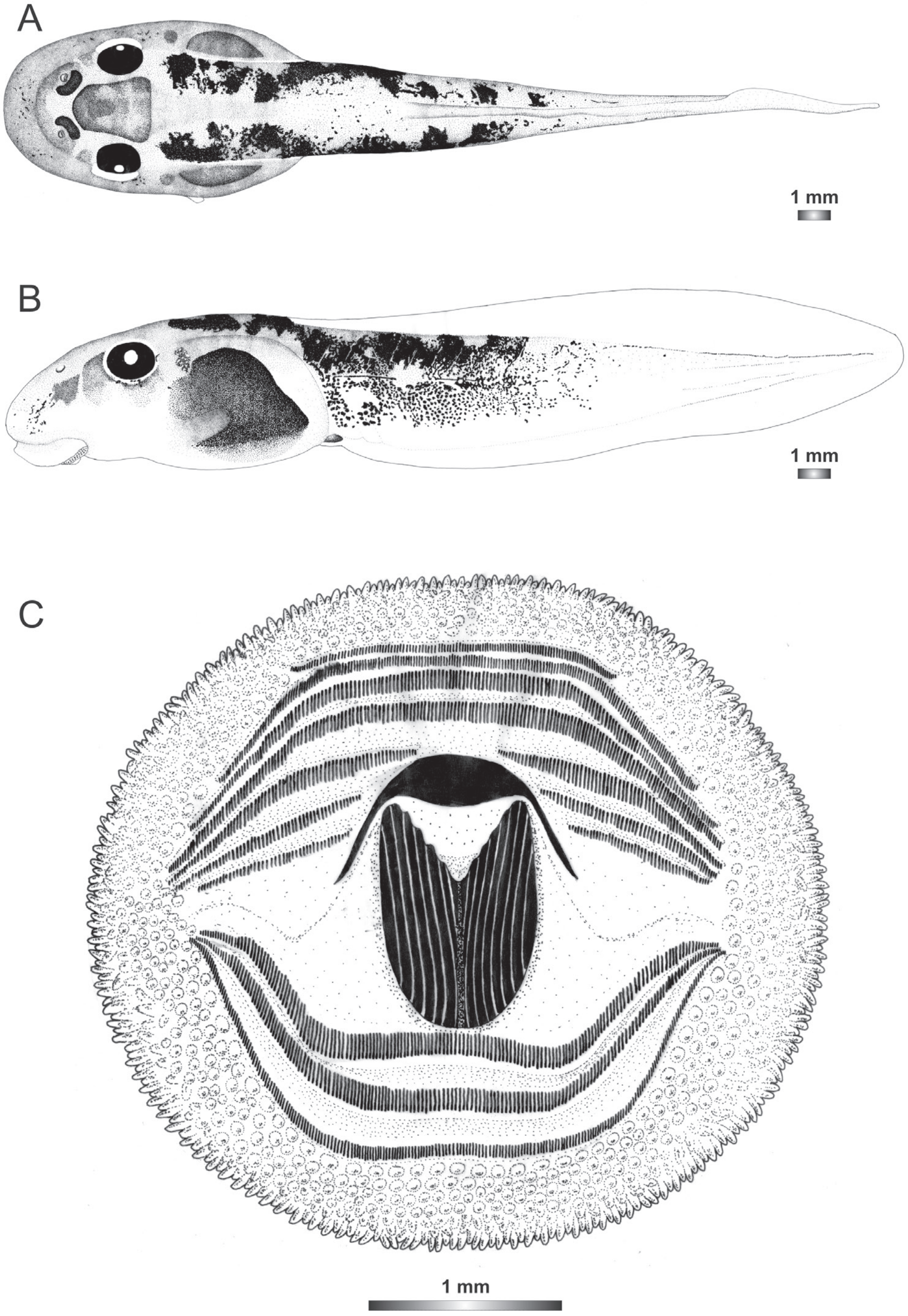

Figure 22. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca52] (ZCMV 13168-ZSM 541/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

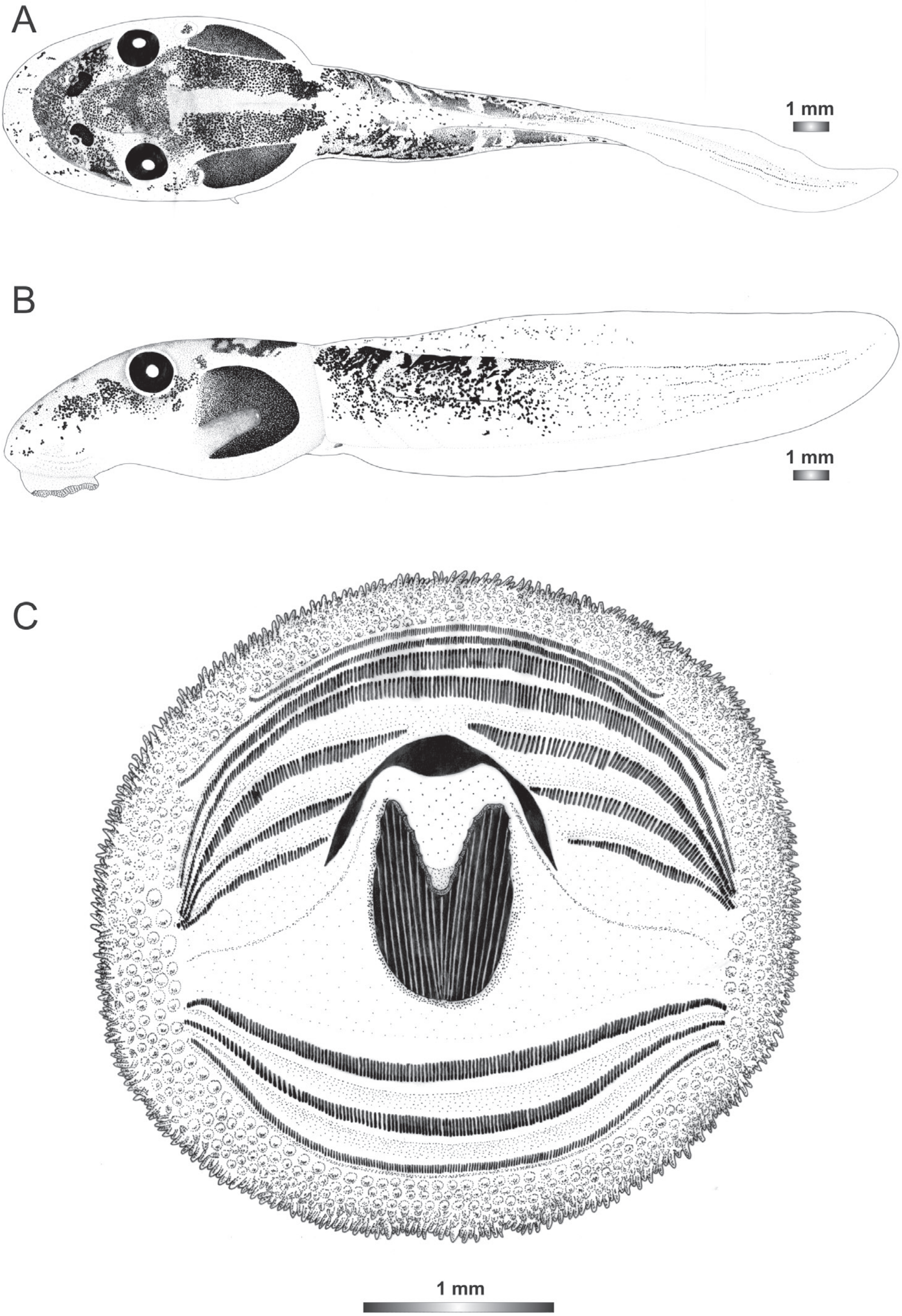

Figure 23. Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca53] (ZCMV 13200-ZSM 573/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

marojezensis [Ca52] by the presence of a distinct lateral clear area surrounding the body (Figure 1). The general pigmentation pattern is similar to that of *B. marojezensis* [Ca26].

Boophis vittatus **Glaw, Vences, Andreone & Vallan, 2001**

Morphological data were assessed in one tadpole (Figures 2 and 24) in developmental stage 29 (field number FGZC 2238, ZSM 1906/2007, BL 7.8 mm, TL 18.5 mm, Genbank accession number JQ518200), from Marojejy National Park - Camp Mantella. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of a *B. vittatus* adult specimen (accession FJ559158) from the same locality. Three other voucher tadpoles of *B. vittatus* are morphologically very similar to the described voucher specimen.

The tadpoles of *B. vittatus* are the smallest tadpoles in this group. They have also a *marojezensis*-like oral disc structure with 289 marginal and 326 submarginal papillae. The tadpoles of this species are provided with a lateral transparent area of the body wall which is more pronounced surrounding the 2/3 anterior of the body. The tail musculature is reticulated like in *B. marojezensis* [Ca51].

Occurrence of strongly rheophilous *Boophis* tadpoles in streams of Ranomafana

In streams of Ranomafana National Park, during the wet season, tadpoles of 44 frog species were found of which five had the morphological characteristics of the strongly rheophilous *Boophis*. These species were found in eleven out of 33 streams. *Boophis andohahela* occurred in eight streams with a mean of 9.9 specimens (min=1 to max=31 specimens), *B. ankaratra* occurred in two streams each with one single specimen, *Boophis marojezensis* [Ca51] was found in seven streams with a mean of 6.3 specimens (1 to 16 specimens), and only a single specimen of *B. schuboeae* was found. The tadpoles of *B. luciae* (named *B.* sp. 17 in Vieites et al. 2009) were found in eight streams with a mean of 12 specimens (1 to 33). During the dry season, 23 species were found of which three belong to the group of strongly rheophilous *Boophis*. Those species were found in 30% of the sampled streams in this season. *B. andohahela* occurred in 23% of the streams with nine specimens on average, *Boophis marojezensis* [Ca51] occurred in 30% of the sampled streams with three specimens on average, and *B. luciae* occurred in 15% of the sampled streams with eight specimens on average.

Breeding site choice

Principal Component Analysis on the habitat variables of the stream and the surrounding forest at Ranomafana resulted in three principal components, explaining

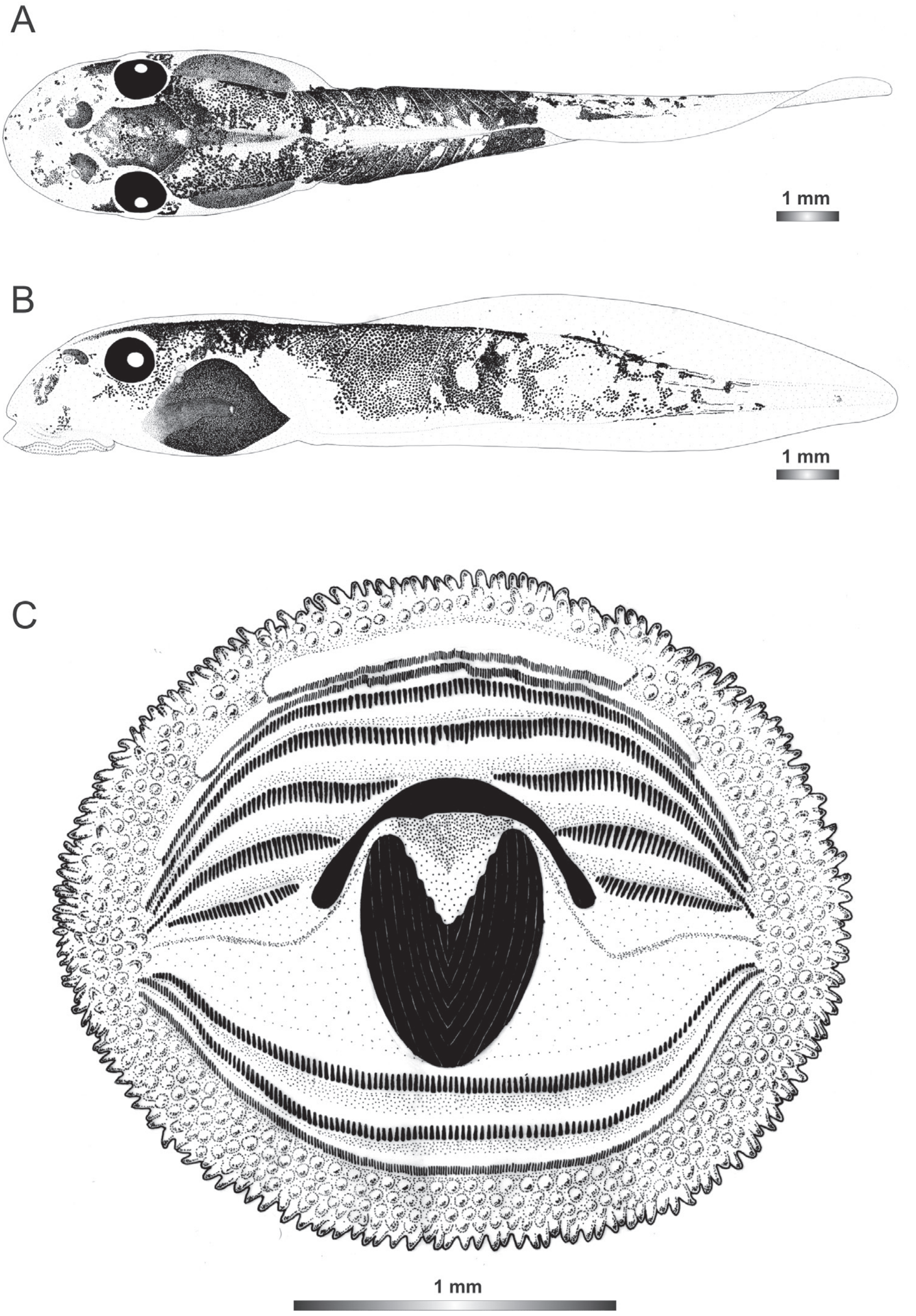

Figure 24. Drawings of the preserved DNA voucher tadpole of *Boophis vittatus* (FGZC 2238-ZSM 1906/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

together 65.5% of the variation in the data. We identified the following habitat variables being well represented (Figure 25): PC1 (33.8%) positive: slope and canopy cover of forest and stream, overhanging vegetation; negative: width and depth of the stream. Also four of the strongly rheophilous tadpole species, *B. ankaratra*, *B. andohahela*, *B. luciae*, and *B. marojezensis* [Ca51] are negatively correlated with this PC. The strongest contributors to PC2 (17.6%) were positive: forest leaf litter depth, stream overhanging plants, trees, and stream canopy cover; negative: slope of forest and stream. *B. andohahela* and *Boophis marojezensis* [Ca51] are negatively correlated with this PC. To PC3 (14.1%), the following variables were positive: number of small trees and shrubs in the forest and overhanging vegetation. Due to its rareness, no correlation of *B. schuboeae* incidence and PCs can be statistically assessed.

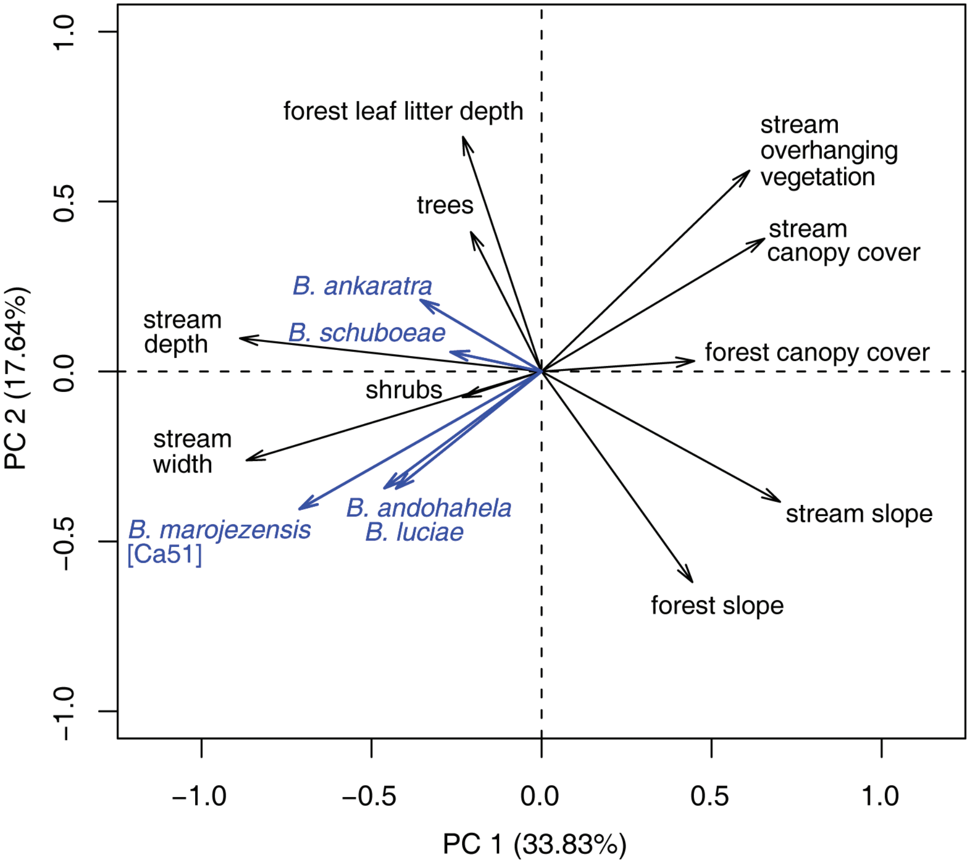

Figure 25. PCA biplot of variables of stream and surrounding habitat as recorded during a tadpole community study in Ranomafana National Park. The five present species of strongly rheophilous tadpoles are included as supplementary variables. Length and direction of vectors can be interpreted as correlations.

Microhabitat choice

Strongly rheophilous *Boophis* tadpoles were found in all microhabitats available in streams of Ranomafana National Park (Figure 26). A considerable amount of specimens was found in microhabitats characterised by fast flowing water and substrates of rock, gravel, and sand which generally do not harbour many tadpoles (own unpublished data). Tadpoles of *B. andohahela* were also relatively often found in slow moving parts of the streams with leaves and sand as substrates. Of the two locally rare species, *B. ankaratra* and *B. schuboeae*, one specimen of each was found in fast rock and fast sand microhabitat, and one specimen in slow rock microhabitat, respectively.

Considering the availability of microhabitats in the streams, Ivlev's electivity index (E, Ivlev 1961) shows that strongly rheophilous *Boophis* do not show a consistent microhabitat preference or avoidance except for “slow gravel” which is avoided by all species, and there is no general difference between the three species (Figure 27); factorial ANO-

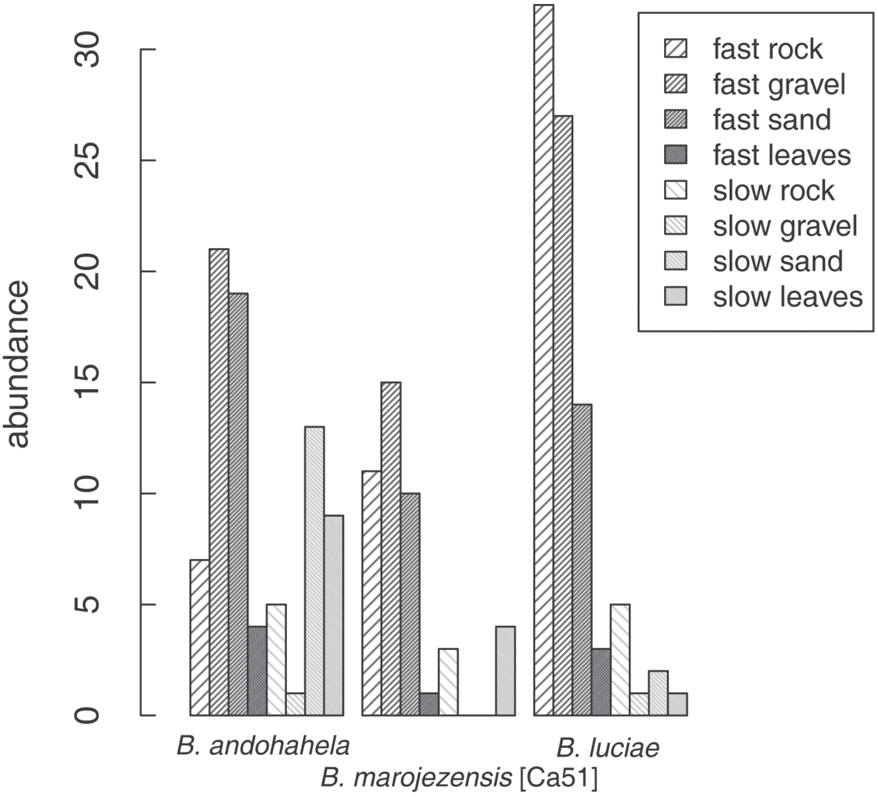

Figure 26. Tadpole distribution across the eight microhabitats (defined using water current and stream substrat) of the three most abundant strongly rheophilous *Boophis* that were sampled in Ranomafana National Park in wet season 2008. *B. andohahela*: N=8 , *B. marojezensis* [Ca51]: N=7, *B. luciae* N=10 with N= the number of streams.

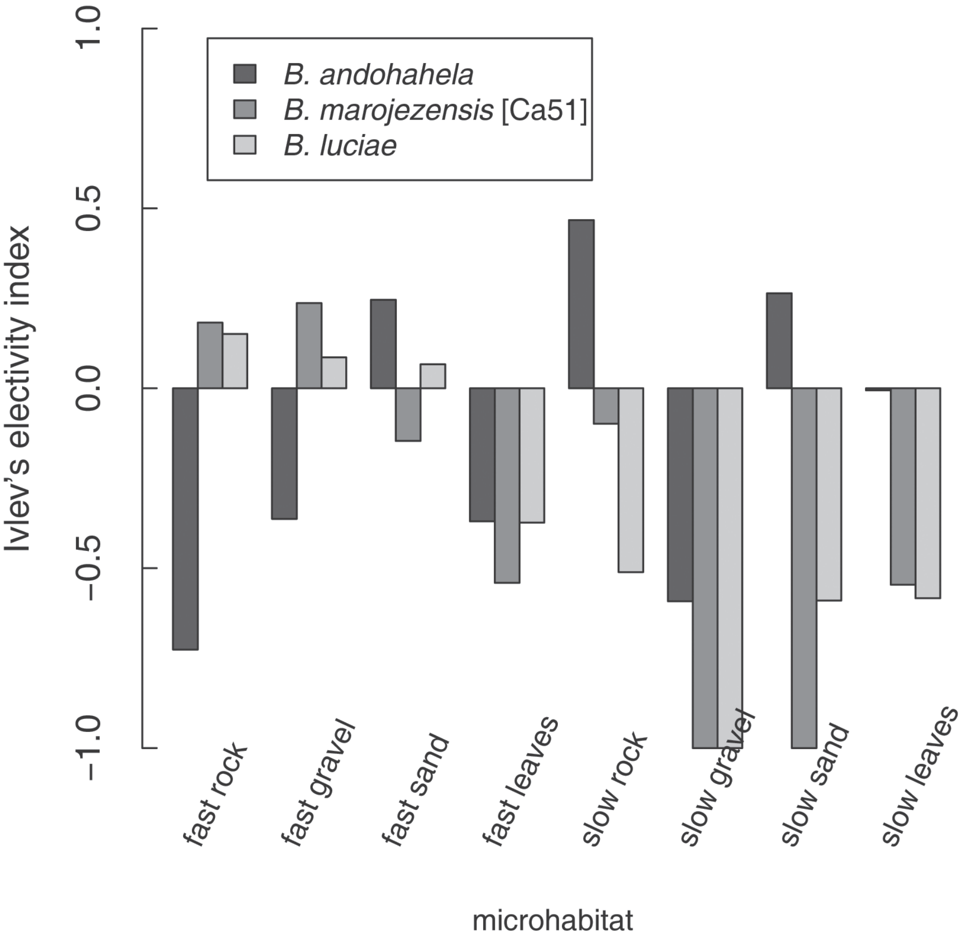

Figure 27. Barplot displaying tadpole microhabitat use of three most abundant *Boophis* species with strongly rheophilous tadpole morphology. Microhabitat use is calculated using Ivlev's electivity index (E, Ivlev 1961) with positive values representing microhabitat preferences and negative values representing microhabitat avoidance. For each species, only streams with at least eight specimens were used for analysis (*B. andohahela*: N=3, *B. marojezensis* [Ca51]: N=2, *B. luciae*: N=4 with N= the number of streams).

VA without interaction term including only streams with at least eight specimens of the respective species; $F_{9,53}=1.716$, $p_{model}=0.108$, $p_{SG}=0.008$, all other p including the factor "species" p>0.26). As interaction terms could not be included in this factorial ANOVA due to overparameterisation, we performed ANOVAs of subsets of the data and found that inter-species differences could only be shown for the microhabitat "fast rock" which is strongly avoided by *B. andohahela* (ANOVA of microhabitat subset; $F_{2,5}=22.6$, $p_{model}=0.003$, $p_{B.\ andohahela}<0.001$) whereas *B. marojezensis* [Ca51] and *B. luciae* were found much more often than *B. andohahela* ($p_{B.\ marojezensis\ [Ca51]}=0.003$, $p_{B.\ luciae}=0.002$). For "slow sand", only for *B. marojezensis* [Ca51] an avoidance could be detected (ANOVA of microhabitat subset; $F_{2,5}=3.829$, $p_{model}=0.098$, $p_{B.\ marojezensis\ [Ca51]}=0.047$), *B. andohahela* and

B. luciae used "slow sand" as much as available ($p_{B.\ andohahela}$=0.427, $p_{B.\ luciae}$=0.105). For all other microhabitats, no significant difference in microhabitat use of species could be detected. However, it should be noted that missing significances can be caused by the number of replicates (streams) which were reduced as we considered only streams with at least eight specimens of the respective species. A graphical evaluation of microhabitat use indicates that non-preferences or non-avoidances are in fact present (Figure 27).

Discussion

Comparisons to previous descriptions of strongly rheophilous *Boophis* tadpoles

Twenty-two strongly rheophilous tadpoles are characterized morphologically in this study, including fourteen tadpoles that are described for the first time and eight other species that had been previously described by other authors. Strongly rheophilous *Boophis* tadpoles have long been known by the work of Blommers-Schlösser (1979), and we here compare her descriptions of *Boophis majori*, *B.* sp., *B. erythrodactylus*, and *B. mandraka* larvae with current knowledge.

The tadpoles of *B. majori* described by Blommers-Schlösser (1979) correspond to those here assigned to the *B. marojezensis* complex, which is reasonable because also the taxonomic concept of *B. majori* of Blommers-Schlösser (1979) included *B. marojezensis,* a species that was only described later by Glaw et al. (2001). Assigning her *B. majori* tadpoles to one of the *B. marojezensis*-like candidate species is supported by their general external morphology with the sinistral spiracle situated on the 3/4 of the body, the well developed caudal musculature, the dark pattern on the body dorsum, the golden ventral side, the oral disc composed by complete small papillae, the lower jaw sheath that is higher than wide, the presence of an upper jaw sheath, and the LTRF of 7(5–7)/3. However, the finding of a dorsal gap of the papillae in some tadpoles is not in accordance with our description, because all *marojezensis*-like tadpoles (*B. marojezensis, B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*) lack a dorsal gap of papillae (data herein and in Raharivololoniaina et al. 2006). We conclude therefore that those tadpoles mentioned by Blommers-Schlösser (1979) might be strongly rheophilous tadpoles from another species, possibly belonging to the *B. albipuctatus* group. Additionally, the relative tail length which is two times of the body length and the situation of the nares close to the eyes of the tadpoles examined by Blommers-Schlösser (1979) do not agree with our data, because all relevant tadpoles in this study have a rather short tail (TAL 166 - 188% of BL) and an opening of the nares that is closer to the snout than to the eyes or in the middle (RN/NP 78 – 103%).

The tadpoles of *Boophis* sp. (Blommers-Schlösser, 1979) are similar to the *B. luteus* group tadpoles described herein according to their general oral disc structure. The LTRF 6(3–6)/3(1) corresponds to those of *B. englaenderi* tadpoles and 6(3–6)/3 to those of *B. englaenderi* [Ca23] tadpoles: This indicates that these tadpoles might belong to two different *Boophis* species. Since *B. englaenderi* and *B. englaenderi* [Ca23] do not occur in the site where Blommers-Schlösser (1979) observed her *Boophis* sp. tadpoles, we hypothesize that those tadpoles belong to species in *B. luteus* group whose larval stages are not yet known.

Tadpoles having narial openings closer to the eyes than to the snout, a sinistral spiracle situated on the 3/4 of the body, a well developed caudal musculature, a rounded oral disc with a LTRF of 7(5–7)/3, a dorsal gap of papillae and a complete jaw sheath were also described and assigned to *Boophis erythrodactylus*, a species of the *B. rappiodes* group, by Blommers-Schlösser (1979). The species identification of those tadpoles, however, is uncertain as already mentioned by Raharivololoniaina et al. (2006): *(1)* all the other species of the *B. rappiodes* group have generalized tadpoles, i.e., *B. rappiodes* as described in Blommers-Schlösser (1979) and Raharivololoniaina et al. (2006), *B. tasymena* and *B. viridis* in Raharivololoniaina et al. (2006), and *B. bottae* in Randrianiaina et al. (2009a), and *(2)* those tadpoles were stated to occur in sympatry with *B. mandraka* tadpoles, and either might just be a variation of *B. mandraka* tadpoles or belong to a closely related species of *B. mandraka* with similar tadpoles. As we encountered several times in our study, the tadpoles of two closely relative species can live sympatrically.

As described by Blommers-Schlösser (1979), the tadpoles of *B. mandraka* have a sinistral spiracle that opens at 2/3 of the body, narial openings closer to the eyes than to the snout, a tail that is two times longer than the body, a well developed caudal musculature, a silvery belly, an almost rounded oral disc with a V-shaped lower sheath and a LTRF of 7(6–7)/3. So far no strongly rheophilous tadpoles with only two interrupted upper keratodont rows have been observed in our study. We have observed in some tadpoles of *B. sibilans* and *B. luciae* that the gap separating the A_5 row is very tight which might be responsible for the false impression of an uninterrupted row.

Tadpoles of *B. andohahela* from Ranomafana were described by Thomas et al. (2006). The general morphology and the oral disc structure of the tadpoles agrees with our specimens, except the keratodont row formula and the presence of a ventral gap of marginal papillae. Thomas et al. (2006) described tadpoles with a LTRF 6(3–6)/3(1), although in our study all tadpoles from the same locality as in Thomas et al. (2006) have a LTRF 6(3–6)/3. This might be caused by the fact that the teeth in the first lower row are very dense, and sometimes it folds in the middle giving the mistaking impression of a gap.

The *B. sibilans* tadpoles from Andasibe that Raharivololoniaina et al. (2006) described agree with our specimen except some minor differences; e.g., the relative width of the oral disc. These differences might be due to the different developmental stages of the tadpoles in the two studies, or by the different methods that have been used for taking the respective measurements.

Glos et al. (2007) described the tadpoles of *B. schuboeae* from Ranomafana and of *B. ankaratra* from Andringitra. The morphology therein is in accordance to the specimens of our study.

B. englaenderi, *B. vittatus* and *B. luciae* were described by Rasolonjatovo et al. (2010). We redescribe these species because of the bad condition of the voucher specimens and/or the lack of some data in the previous descriptions. The same tadpole specimen of *B. englaenderi* from Marojejy National Park was redescribed to facilitate the comparison to the other *B. luteus* group tadpoles. We furthermore described the tadpoles of *B. vittatus* and *B. luciae* from the same locality based on new voucher specimens because of the bad condition of the vouchers used in Rasolonjatovo et al. (2010).

Morphological differences among tadpoles of closely related species

As decribed by Blommers-Schlösser (1979), defined by Raharivololoniaina et al. (2006), confirmed by Glos et al. (2007) and observed herein, strongly rheophilous tadpoles are typical stream-inhabiting organisms, and are characterized by a narrow and flat elongated body, a well developed caudal musculature, a wide oral disc with many small papillae that are either complete or interrupted by a dorsal gap, a rather small and ribbed (i.e., composed of a series of fused columns) lower jaw sheath, many upper keratodont rows with at least the two first being uninterrupted and three lower keratodont rows of which in most of the species the first one is uninterrupted.

This type of tadpoles can be found in different *Boophis* species groups: *B. luteus* group, *B. albipunctatus* group, *B. mandraka* group, and *B. majori* group (its occurrence in the *B. rappiodes* group is in need of confirmation). As described by Blommers-Schlösser (1979) and Schmidt et al. (2008), also *B. williamsi* (*B. microtympanum* group) has an enlarged oral disc (ODW 90% of BW, pers obs.) with a LTRF of 8(3–8)/3. However, we did not consider this species in our study because *(1)* this tadpole has a generalized oral disc structure (jaw sheaths, papillae and keratodonts) and *(2)* all the other strongly rheophilous tadpoles have a rather small size (BL 5.9 – 13.5 mm, TL 12.7 – 27.1 mm, in Gosner stages 25 – 36) compared to the montane *B. wiliamsi* tadpoles (BL 25.5 mm and TL 71.7 mm in Gosner stage 36).

Within the main groups of morphologically similar tadpoles, some can be very similar, but usually there are morphological details to differentiate them, whether in the external morphology or in the oral disc configuration; i.e., tadpoles that are very similar in external morphology can be differentiated in oral disc structure and vice versa:

(1) Three tadpoles belonging to the *Boophis luteus* group (*B. englaenderi, B. englaenderi* [Ca23], and *B. andohahela*) look alike in external morphology but can be differentiated easily by their keratodont row formula. Of these, *B. englaenderi* and *B. englaenderi* [Ca23] occur syntopically. The tadpoles of *B. englaenderi* [Ca23] can be distinguished from those of *B. englaenderi* by their relative tail length, by their pigmentation pattern and mainly by their oral disc structure (LTRF and number of papillae).

(2) In the *B. albipunctatus* group, *B. ankaratra*, *B. schuboeae*, *B. sibilans* and *B. luciae* are similar. *B. ankaratra* and *B. schuboeae* occur sympatrically, and they can be differentiated by the presence of dark pigmented bands on the tail muscle in *B.*

schuboeae, and also by the absence of papillae on the lateral area where the oral disc folds in *B. schuboeae*. *B. sibilans* and *B. luciae* differ by the presence of a dark bridge which connects the dark sections on the tail muscle in *B. luciae.*

(3) All tadpoles known from the species of the *B. mandraka* group have a similar oral disc configuration, characterized by the absence of the upper jaw sheath and a LTRF of 8(6–8)/3. The tadpoles of *B. sambirano* and *B. mandraka* [Ca46] are very similar, except that *B. mandraka* [Ca46] has the narrowest dorsal gap of marginal papillae. The fact that these two tadpoles live allopatrically can help also to identify them. Five species of this group are distributed in close proximity in the North of Madagascar. Of these, *B. sambirano* [Ca47] and *B. sambirano* [Ca48] are sympatric, and can be differentiated by the patched *vs.* spotted pattern on the tail. *B. sambirano* [Ca49] and *B. sambirano* [Ca50] live also sympatrically. *B. sambirano* [Ca49] can be distinguished to the three other species by its generally dark coloration pattern, the ovoidal form of the body in dorsal view and the wide inter-orbital distance. *B. sambirano* [Ca50] can be differentiated by the intensity of the golden pigments which may cover the whole body and overlay the dark pigment in some specimens. *B. mandraka* [Ca38] is very typical by its weakly expressed pigmentation.

(4) Two tadpoles belonging to two different groups, *B. albipuncatus* (*B. albipunctatus* group) and *B. mandraka* [Ca38] (*B. mandraka* group) are similar in their weak expression of pigmentation, but they can easily differentiated by their oral disc morphology.

(5) Two cases of similarity are also found in *B. majori* group tadpoles. *B. marojezensis, B. marojezensis* [Ca26], *B. marojezensis* [Ca53] and *B. vittatus* are very similar in the presence of a clear, not pigmented lateral area surrounding the body, and in the tail pigmentation pattern. The fact that several of these species can occur sympatrically increases also the chance to confound them. On the other hand, the tadpoles of *B. marojezensis* [Ca51] and *B. marojezensis* [Ca52] are similar in the absence of a lateral clear area surrounding the body, and in their general pigmentation pattern. Only the tadpole of *B. marojezensis* [Ca25] is easily distinguishable by the presence of clear and more or less rounded patches on the tail muscle. As the three *B. marojezensis*-like tadpoles, *B. marojezensis*, *B. marojezensis* [Ca25] and *B. marojezensis* [Ca26], live syntopically in Marojejy National Park, *B. marojezensis* [Ca25] tadpoles will not be confounded with those of the two other species.

Morphological clusters of strongly rheophilous *Boophis* tadpoles

Analyzing the structure of the oral disc of all these tadpoles allows classifying them into three clusters:

(1) The first cluster including three *B. luteus* group tadpoles is characterized by a moderately wide to very wide (ODW 56 to 84% of BW), non emarginated,

ventrally positioned and oriented oral disc, which has an anterior margin connected directly to the snout, two uninterrupted upper rows of keratondonts (LTRF is 6(3–6)/3(1) for *B. englaenderi* but 6(3–6)/3 for the tadpoles of *B. englaenderi* [Ca23] and *B. andohahela*); a very long A_1 (82 to 90% of ODW); a high number of keratodonts in A_1 (220 to 301), totally keratinized; typically narrow to moderate sized jaw sheaths (JW 31 to 46% of ODW) with a very short medial convexity (MCL 0.04 to 0.11% of JW); a wide to very wide dorsal gap of papillae (DG 67 to 85% of BW); a low number of submarginal papillae (33 to 94) and a medium number of marginal papillae (101 to 175); a high positioned eye (EH 69 to 85% of BH) that is situated not far from midbody (SE 32 to 39% of BL); very high positioned nares (NH 57 to 82% of BH) that are situated below or at eye level (NH 82 to 97% EH) and closer to the snout than to the eye (RN 60 to 92% of NP); a short tail (TAL 155 to 183% of BL), and a developed caudal musculature.

(2) The second cluster is characterized by a wide to hyper-wide (ODW 74 to 108% of BW) non emarginated, ventrally positioned and oriented oral disc with an anterior margin separated from the snout by a shallow crevice or free; four or five uninterrupted upper rows of keratondonts giving a LTRF of 7(5–7)/3 or 8(6–8)/3); a short to moderately sized A_1 (21 to 59% of ODW); a low to medium number of keratodonts in A_1 (95 to 241), totally keratinized; U-shaped, ribbed narrow upper jaw sheaths (JW 30 to 34% of ODW which can present a small medial convexity or not); a U-shaped and "ribbed" lower sheath, a moderately wide to very narrow dorsal gap of papillae (DG 14 to 59% of BW); a medium to high number of marginal papillae (148 to 377), many submarginal papillae (190 to 368); high to very high positioned eyes (EH 71 to 84% of BH) that are situated closer to the snout than to midbody (SE 35 to 49% of BL), high to very high positioned nares (NH 64 to 92% of BH) that are situated below or above the eye level (NH 86 to 112% of EH) and closer to the eye than to the snout (RN 107 to 194% of NP); a short to very short tail (TAL 146 to 184% of BL); and a developed caudal musculature. Tadpoles of the *B. albipuncatus* group (*B. schuboeae*, *B. ankaratra*, *B. albipunctatus*, *B. sibilans*, and *B. luciae*) and *B. mandraka* group (*B. sambirano*, *B. mandraka* [Ca38], *B. mandraka* [Ca46], *B. sambirano* [Ca47], *B. sambirano* [Ca48], *B. sambirano* [Ca49], and *B. sambirano* [Ca50]) belong to this cluster. All *B. mandraka* group tadpoles lack a keratinized upper jaw sheath.

(3) The third cluster is characterized by a wide (ODW 68 to 79% of BW) non emarginated, ventrally positioned and oriented oral disc without a dorsal gap of papillae and with the anterior margin being free from the snout; four uninterrupted upper keratodont rows (LTRF 7(5–7)/3); a moderately sized A_1 (45 to 52% of ODW); a medium number of keratodonts in A_1 (126 to 235); a totally keratinized upper jaw sheath (JW 30 to 38% of ODW) without medial convexity; a U-shaped and ribbed lower sheath, many submarginal (222 to 318) and marginal

(206 to 522) papillae; high positioned eyes (EH 68 to 80% of BH) that are situated closer to midbody (SE 35 to 49% of BL); very high positioned nares (NH 68 to 80% of BH) that are situated below eye level except for *B. vittatus* and *B. marojezensis* [Ca25] (NH 89 to 101% of EH) and closer to the snout for the most (RN 78 to 109 % of NP); a very short to short tail (TAL 140 to 188% of BL), and a developed caudal musculature. Tadpoles of the *B. majori* group (*B. marojezensis, B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*) belong to this cluster.

Although we lack at this time an explicit and well supported phylogenetic hypothesis for the relationships among all these species of *Boophis*, the morphological characters of these three morphological clusters can serve to develop a possible evolutionary scenario of the origin of the specializations in strongly rheophilous *Boophis* tadpoles, departing from the structures in more generalized *Boophis* tadpoles. All of the latter are characterized by having one (the first) uninterrupted upper keratodont row and one (the first) interrupted lower keratodont row, typically smooth (non-ribbed) jaw sheaths and a medial convexity in the upper jaw sheath (see Raharivololoniaina et al. 2006, Randrianiaina et al. 2009a, b, and Rasolonjatovo et al. 2010). Specialization to a strongly rheophilous life thus involves *(1)* reduction of the size of the jaw sheaths correlated with *(2)* the disappearance of the medial convexity, *(3)* reduction of the size of the dorsal gap of marginal papillae, *(4)* reduction of the length of the row A_1, *(5)* reduction of the number of keratodonts in A_1, compensated by an increase of the number of *(6)* marginal and *(7)* submarginal papillae and *(8)* of the uninterrupted upper keratodont rows.

The decrease of the size of the jaw sheaths may provoke the fading of its medial convexity on one hand and leaves a place for many dorsal and lateral, even ventral submarginal papillae, and new uninterrupted upper keratodont rows on the other hand. Also, the reduction of the size of the dorsal gap leads to a higher number of marginal papillae. The development of many dorsal marginal papillae reduces the area available for the first upper keratodont row and thus may cause the reduction of its length, which in turn leads to the decrease of the number of the teeth. However, the loss of the upper jaw sheath in all species and candidate species of the *B. sambirano* complex is still unclear. This characteristic is neither caused by a fixation artifact nor by the transportation of the specimens because we observed it already in the living tadpoles in the field (Figure 29), and because it is consistent within series. The absence of the upper jaw sheath was found even in young tadpoles (Gosner 25) indicating that it occurs very early in larval development. It remains to be tested (e.g., by a study on embryonic development), however, if this structure never develops, or is initially formed but then disappears at some early developmental stage.

Ecomorphological guilds in *Boophis* tadpoles

A magnitude of descriptions of the larval stages of Madagascan frogs have been recently published (Andreone et al. 2002, Glos and Linsenmair 2005, Raharivololoniaina et al. 2003, 2006, Thomas et al. 2005, 2006, Altig and McDiarmid 2006, Vejarano et al. 2006a, b, c, Glos et al. 2007, Schmidt et al. 2008, 2009a, b, Grosjean and Vences 2009, Jovanovic et al. 2009, Rasolonjatovo et al. 2010, Grosjean et al. 2006, 2007, 2011a, b, Randrianiaina et al. 2007, 2009a, b, 2011a, b, c). While some of them merely intended to increase general knowledge on Madagascan tadpoles, others attempted to classify the tadpoles into ecomorphological guilds. For *Boophis* tadpoles, Raharivololoniaina et al. (2006) tried to define three guilds, named A, B and C, mainly based on three variables: relative width of oral disc, number of inframarginal papillae, and number of keratodonts on the first anterior row. As already mentioned by Randrianiaina et al. (2009a), these guilds were not intended to replace nor to refine the guilds of Altig and Johnston (1989), but to achieve a complementary, more quantitative classification that would better fit the variation of the *Boophis* tadpoles studied. Moreover, the criteria chosen by Rahavolololoniaina et al. (2006) were few and some of those that Altig and Johnston (1989) used do not exist in *Boophis* tadpoles (Randrianiaina et al. 2009a). Therefore, a comprehensive definition of adequate guilds for Malagasy tadpoles will require the consideration of numerous new variables without omitting those that have been used before. In this process it is important to notice first the presence or absence of one component (e.g., jaw sheath and keratondont) and then its configuration (e.g., totally or poorly keratinized sheaths, density of papillae; Randrianiaina et al. 2011a).

According to Altig and Johnston (1989), three different guilds might correspond to *Boophis* tadpoles. The clasping tadpoles have a dorsal gap of marginal papillae, commonly five keratodont rows (but as numerous as 8/8), usually with anterior rows that are more numerous than posterior rows (e.g., 9/3), and a globular to slightly depressed body. They inhabit medium to slow water currents and the maintenance of their position in the water current with the help of the oral disc is of minor importance. The adherent tadpoles have small and complete marginal papillae, and a LTRF of commonly 2/3. They inhabit faster flowing water than clasping tadpoles, their position maintenance via the oral disc is common to continuous, and their body is often depressed. The suctorial tadpoles have a depressed body, small and complete marginal papillae, and a LTRF from 2/3 to a maximum of 17/21. They inhabit even faster running waters than the clasping and adherent tadpoles, and their position maintenance via their oral disc is continuous.

In this study, no new guild names are defined, but we suggest to adapt in a preliminary way the guilds already defined by Altig and Johnston (1989).

(1) We do not consider the *B. luteus* group tadpoles truely strongly rheophilous, due to their more generalized and intermediate characteristics. These tadpoles (*B. englaenderi, B. englaenderi* [Ca23], *B. andohahela*) can possible be considered to be part of the "clasping" guild.

(2) The first guild of strongly rheophilous tadpoles, here considered as "adherent", is the second category of tadpoles classified in the previous section which is composed by the tadpoles of the *B. albipuncatus* group (*B. schuboeae*, *B. ankaratra*, *B. albipunctatus*, *B. sibilans*, and *B. luciae*) and the *B. mandraka* group

(*B. sambirano*, *B. mandraka* [Ca38], *B. mandraka* [Ca46], *B. sambirano* [Ca47], *B. sambirano* [Ca48], *B. sambirano* [Ca49], and *B. sambirano* [Ca50]), because they inhabit faster running water and the maintenance of the position in the water via their oral disc is common to continuous. This guild is characterized mainly by the presence of a dorsal gap of papillae and two typical LTRF-s which are 8(5–8)/3 and 8(6–8)/3. All *B. mandraka* group tadpoles lack an upper jaw sheath, while this structure is present in the *B. albipuncatus* group tadpoles.

(3) The second guild that we define as "suctorial" is the third category of tadpoles classified in the previous section which is composed of all *B. marojezensis*-like tadpoles (*B. marojezensis, B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*). They probably inhabit faster running water and maintain continuously their position in the water with the help of their oral disc because of the complete state of the papillae that they have. This guild is characterized by the absence of a dorsal gap of papillae and a LTRF of 7(5–7)/3.

Habitat selection and ecology of strongly rheophilous *Boophis* tadpoles

In the tropical rainforest of Ranomafana National Park, strongly rheophilous *Boophis* tadpoles occur throughout the whole year (own unpublished data) with clearly higher abundances in the wet season. Whereas some species are relatively common (e.g., *B. marojezensis* and *B. luciae*), others are locally extremely rare (e.g., *B. ankaratra*, *B. schuboeae*). In this area, strongly rheophilous *Boophis* do neither include the most common tadpoles species nor is the group itself as common as other groups (Grosjean et al. 2011). Species of this group choose larger, open, slowly running streams for breeding (Figure 28); small streams with high slope and a dense vegetation cover are generally avoided. This is generally true for all strongly rheophilous species studied in Ranomafana National Park. The latter kind of stream might be avoided as they are less attractive to adults than large streams, which provide more space without the risk of egg and tadpole predation by fishes. Small streams might also be characterised by reduced food availability, e.g., due to reduced periphyton growth as a result of high vegetation coverage (Mallory and Richardson 2005; Altig et al. 2007). This actually describes the expected pattern for most tadpoles in Madagascan rainforest streams and can also be observed, e.g., for tadpoles of the *Mantidactylus* subgenus *Ochthomantis*, which are characterised by reduced oral disc structures (Randrianiaina et al. 2011a). In contrast, the also specialized funnel mouthed tadpoles of *Mantidactylus* subgenus *Chonomantis* do not follow this pattern, as for some species no prediction of occurrence by habitat characteristics is possible and some species (e.g., *Mantidactylus opiparis*) prefer combinations of habitat characteristics that are unfavourably represented in our PCs (Grosjean et al. 2011a).

Figure 28. Pictures showing tadpole capture sites inside a primary forest in Ranomafana National Park (**A** in Fompohonina river, **B** in Piste E 100 stream), and outside the forest (**C** in Anjingo river and **D** in Ankijagna Lagnana).

Within the streams, however, strongly rheophilous *Boophis* tadpoles are quite outstanding regarding their microhabitat choice compared to other abundant and well observed tadpole groups. This is especially true for two of the most common of these species, *B. marojezensis* [Ca51] and *B. luciae*, and less pronounced for *B. andohahela*, consistent with the more generalized oral disc structure of this latter species. Whereas we could not show true preferences for fast running sections, we could at least show that a considerable number of specimens are indeed using these faster parts of the streams. This clearly separates these tadpoles from other abundant groups (Grosjean et al. 2011a, Randrianiaina et al. 2011a), and most likely reflects the morphological specialisations of oral disc, body, and tail to withstand the current.

Their large ventral oral disc allows attaching on substrate (Figure 29) such as rocks and gravel, and the presence of numerous short papillae presumably aids in forming a tight seal between the oral disc and the irregularities of substrate (Altig and McDiarmid 1999). Also, their relatively small body size and well developed caudal musculature probably allows a good locomotory performance in strong current.

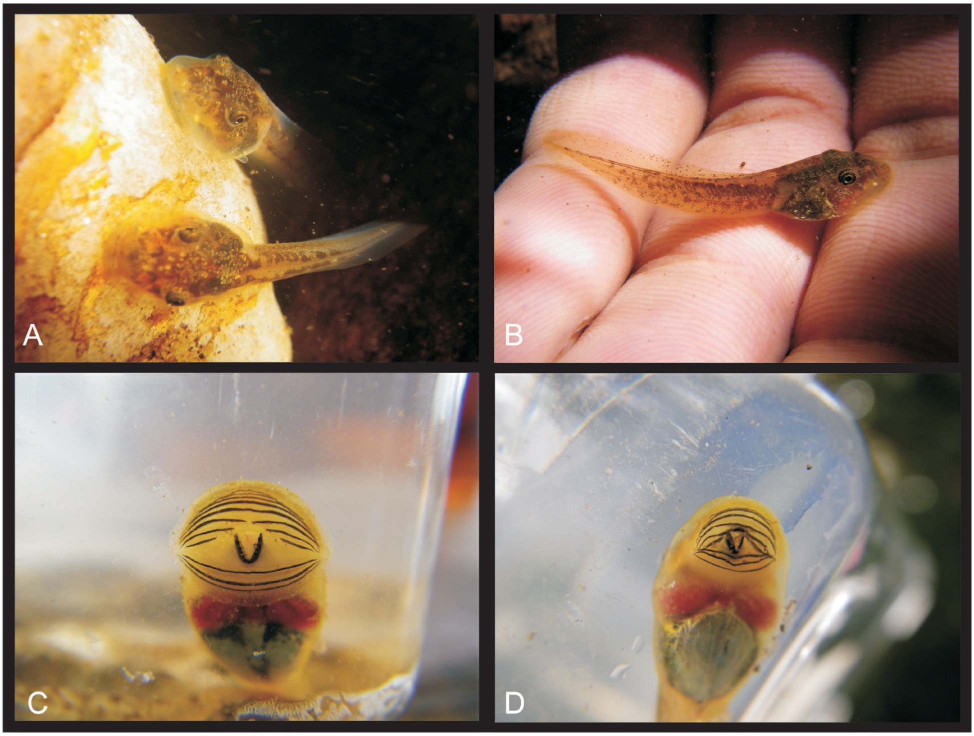

Figure 29. Photographs in life of strongly rheophilous *Boophis* tadpoles: **A** and **B** Underwater pictures of *B. sambirano*-like tadpoles (from Antevialambazaha - Tsaratanana Integral Reserve) **C** and **D** Oral disc of *B. sambirano* [Ca48] (ZCMV 13109 -ZSM 485/2010) and *B. marojezensis* [Ca52] (ZCMV 13169-ZSM 542/2010) (from Ambinanitelo) fixing on the sides of an aquarium.

Reverse taxonomy and high cryptic species diversity of *Boophis*

As already demonstrated by Randrianiaina et al. (2011a), reverse taxonomy, initially defined for unicellular organisms and invertebrates, can also be applied to better studied groups such as vertebrates. Herein we confirm the usefulness of this method by finding numerous divergent tadpole DNA sequences. Twelve candidate species are defined in this study by the divergent DNA sequence of the tadpoles in comparison with the sequences of all species and candidate species previously known by adult specimens. To evaluate the status of such genetically divergent specimens, it is important to evaluate whether (1) the genetic divergence is correlated with other characters, e.g., consistent morphological differences, and (2) whether these consistently differentiated groups may furthermore occur in sympatry, which then suggests they are reproductively isolated evolutionary lineages, and thus, distinct species. We could indeed find such a situation in three pairs of species, and thus can flag several of the newly discovered genealogical lineages as confirmed candidate species (Vieites et al. 2009):

(1) *B. englaenderi* [Ca23] lives syntopically with *B. englaenderi,* and these two forms show clear and constant differences genetically and in larval morphology, as described above, including characters of the oral disc, relative tail length, and coloration.

(2) In the *B. mandraka* group, *B. sambirano* [Ca49] tadpoles are very deviant and can easily be differentiated by coloration and the position of the eyes from the lineage *B. sambirano* [Ca50] occurring at a nearby locality in the same stream.

(3) *B. marojezensis* [Ca25] is very distinct by the presence of more or less rounded patches on the posterior half of the tail musculature which distinguishes it from the two syntopic forms, *B. marojezensis* and *B. marojezensis* [Ca26].

As a conclusion, this extraordinary and surprising diversity of *B. marojezensis*-like and *B. sambirano*-like candidate species especially in northern Madagascar probably indeed reflects a high number of yet undescribed species, and claims for a biogeographic and evolutionary explanation. It further confirms that stream-breeding frogs apparently show a higher geographical structuring of their diversity (e.g., Inger et al. 1974; Vences et al. 2002). An in-depth revision of these frogs is necessary to understand this diversity and its taxonomic relevance, and needs to be based on an integrative approach assessing their bioacoustic, and nuclear genetic divergence, focusing on sympatric occurrences which we expect to be particularly informative regarding the isolation mechanisms between these lineages.

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Appendix 1

Table 3–5. (doi: 10.3897/zookeys.178.1410.app1) File format: Excel spreadsheet (xls).

Explanation note: Appendix I contains Table 3–5.

Citation: Randrianiaina RD, Strauß A, Glos J, Vences M (2012) Diversity of the strongly rheophilous tadpoles of Malagasy tree frogs, genus *Boophis* (Anura, Mantellidae), and identification of new candidate species via larval DNA sequence and morphology. ZooKeys 178: 59–124. doi: 10.3897/zookeys.178.1410.app1

Appendix 2

Morphological description of the rheophilous *Boophis* tadpoles. (doi: 10.3897/zookeys.178.1410.app2) File format: MS Word document (.doc).

Explanation note: Appendix II contains complete morphological description of the rheophilous *Boophis* tadpoles.

Citation: Randrianiaina RD, Strauß A, Glos J, Vences M (2012) Diversity of the strongly rheophilous tadpoles of Malagasy tree frogs, genus *Boophis* (Anura, Mantellidae), and identification of new candidate species via larval DNA sequence and morphology. ZooKeys 178: 59–124. doi: 10.3897/zookeys.178.1410.app2