Zoosystematics and Evolution

90 (2) 2014

http://zse.pensoft.net

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Scope

Zoosystematics and Evolution (formerly Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologische Reihe) edited by the Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University Berlin is an international, peer-reviewed, life science journal, devoted to whole-organism biology. It mainly publishes original research and review articles in the field of Metazoan taxonomy, biosystematics, evolution, morphology, development and biogeography at all taxonomic levels. Its scope encompasses primary information from collection-related research, via taxonomic descriptions and discoveries, revisions, annotated type catalogues, aspects of the history of science, and contributions on new methods and principles of systematics. Entomological papers will also be accepted for review, but authors should first consider submission to the Deutsche Entomologische Zeitschrift. Articles whose main topic is ecology, functional anatomy, physiology, or ethology are only acceptable when of clear systematic or evolutionary relevance and perspective. Review articles and contributions to a discussion forum are welcome, but authors are asked to contact the editors beforehand.

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- Results
- Discussion
- Acknowledgements
- References
- Tables with captions
- Figure captions

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In Focus

The cover picture shows North-American Unionida of conservation concern in the Museo di Zoologia dell’Università di Bologna, Italy.

See paper of Albano P et al. Natural history museums as repositories of endangered diversity: the case of the United States Unionida in the Museo di Zoologia dell’Università di Bologna
Zoosystematics and Evolution
A Bulletin of Zoology since 1898

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Biological Abstracts® (Thompson ISI)
BIOSIS Previews® (Thompson ISI)
Cambridge Scientific Abstracts (CSA/CIG)
Web of Science® (Thompson ISI)
Zoological Record™ (Thompson ISI)
Elasmopus yucalpeten sp. n. (Crustacea, Amphipoda, Maeridae) from the northern Yucatan coast, with a key for the genus in the Gulf of Mexico and biogeographic comments

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Abstract

A new amphipod species of the genus Elasmopus Costa, 1853 is described and illustrated based on material collected in a harbor on the northern Yucatan coast, southern Gulf of Mexico. Elasmopus yucalpeten sp. n. is recognized from its congeners by a two-articulate accessory flagellum, a group of long robust setae on the anterodistal margin of the gnathopod 2 basis, a distomedial concave portion on palm of gnathopod 2 propodus, long setae on basis posterior margin of pereopods 5–7, and an entire telson. The differences among closely related species are pointed out and they are compared with the new species. An identification key to species of the genus Elasmopus in the Gulf of Mexico and biogeographic comments at the regional and global scales are also provided.

Key Words

Peracarida
Senticaudata
species diversity
taxonomy
zoogeography
Caribbean Sea
Atlantic Ocean
Pacific Ocean
Indian Ocean

Introduction

The genus Elasmopus Costa, 1853 is the most diverse genus in the family Maeridae Krapp-Schickel, 2008, roughly with 101 of the 328 species in the family distributed worldwide in temperate and tropical seas (Ahyong et al. 2011, Vader and Krapp-Schickel 2012). The species are mostly found on the continental shelf (≤ 200 m depth), mainly associated with macrophytobenthos (e.g. algae, marine angiosperm) and secondarily with epifauna (e.g. anemone-hermit crab symbiosis, sponges, zoanthids) (Souza-Filho and Senna 2009, Vader and Krapp-Schickel 2012).

Morphologically, two groups of species inside Elasmopus have been recognized by Vader and Krapp-Schickel (2012): the pectenicrus-group and the rapax-group. The first one consists of most of the species with castelloserrate posterior margins on the basis in one or more pairs of the last pereopods (pereopods 6–7); the second group consists of those species with long setae and crenulate or smooth posterior margins on the basis of the same pereopods. Regionally, 19 nominal species of the genus Elasmopus have been reported in the western Atlantic, 11 in the tropical western Atlantic and eight in the Gulf of Mexico (Ortiz et al. 2007, LeCroy et al. 2009, Gable et al. 2010, Vader and Krapp-Schickel 2012). The reported species in the Gulf of Mexico are: Elasmopus balkomanus Thomas & Barnard, 1988, Elasmopus lemaitrei Ortiz & Lalana, 1994, Elasmopus levis (Smith, 1873), Elasmopus cf. magnispinatus Kunkel, 1910, Elasmopus pectenicrus (Bate, 1862), Elasmopus pocillimanus (Bate, 1862), Elasmopus
rapax Costa, 1853 and Elasmopus thomasi Ortiz & Lala-
nà, 1994. Two other generic species (Elasmopus sp. A and
Elasmopus sp. B) have been described regionally from the
southeastern Gulf of Mexico (Florida) by LeCroy (2000),
but no full description has been yet provided. Now, in the
present paper, Elasmopus yucalpeten sp. n. is described
from collected specimens in the northern Yucatan coast,
southeastern Gulf of Mexico, thus increasing the diversity
of the genus in the Gulf of Mexico to nine species. The
new species belongs to the rapax-group.

Material and methods

The samples were hand collected in the Yucalpeten harbor
as part of a survey in the northern Yucatan coast, south-
eastern Gulf of Mexico. The collected material was passed
through a 0.5 mm sieve, fixed in 10% formalin buffered
with seawater; then it was washed in freshwater, sorted
and preserved in 70% ethanol. Specimens were dissect-
ed in glycerine under a dissecting microscope and illustra-
tions were made under a compound microscope with camera lucida. The description, remarks, and morpholog-
ical comparison follow the style of Appadoo and Myers
(2003), Souza-Filho and Senna (2009), and Hughes and
Lowry (2011). Type material is deposited in the “Colec-
tión de Invertebrados Bentónicos de Yucatán, Cinvestav
(CYMX)” and in the “Colección de Referencia de Ben-
tos Costero, El Colegio de la Frontera Sur (ECOSUR)”.
The following abbreviations are used in the figures: AF,
accessory flagellum; EP, epimeron; G, gnathopod; H, hab-
itus; LL, lower lip; MD, mandible; MP, maxilliped; MX,
maxilla; P, pereopod; T, telson; U, uropod; UL, upper lip.

Systematics

Order Amphipoda Latreille, 1816
Family Maeridae Krapp-Schickel, 2008
Genus Elasmopus Costa, 1853

Elasmopus yucalpeten sp. n.
http://zoobank.org/A76BA32A-4BC1-424E-9359-9696A281342F
Figures 1–4

Type material. Holotype male (dissected and drawn), 6.6
mm, Yucalpeten harbor, Yucatan, Mexico, 21°16.667’N,
89°42.001’W, 24 August 2010, on wood dock with algae,
coll. C. E. Paz-Ríos, CYMX-1-EY. Paratypes: one fe-
male (dissected and drawn), 6.2 mm, data as for holotype,
CYMX-2-EY; four males, four females, data as for holo-
type, ECOSUR0164; six males, Yucalpeten harbor, Yucatan,
Mexico, 21°16.667’N, 89°42.001’W, 26 June 2012, on rock
with algae, intertidal, coll. C. E. Paz-Ríos, CYMX-3-EY.

Other material. Twenty-six males, 49 females, seven unsexed,
data as for holotype, CYMX-4-EY.

Type locality. Yucalpeten harbor, Yucatan, Mexico.

Diagnosis. Body dorsally setose. Antenna 1 long. Access-
ory flagellum two-articulated. Eyes large, ovate. Group
of five long robust setae on basis anterodistal margin of
gnathopod 2. Medial concave portion on palm of gnatho-
pod 2 propodus. Long setae on basis posterior margin of
pereopods 5–7. One basofacial robust seta on peduncle of
uropod 1. Telson entire.

Description. Based on holotype male, 6.6 mm (CYMX-
1-EY). Body with groups of one to three setae on head,
pereon and pleon. Eyes large, ovate. Lateral cephalic lobe
broad, rounded, anteroventral margin with notch/slit. An-
tenna 1 reaching beyond half of body, longer than antenna
2 (about 2 times the antenna 2 length); peduncular article
1 subequal in length to article 2, without robust setae on
posterior margin; article 2 longer than article 3 (about 1.4
times the article 3 length); flagellum with 27 articles; ac-
cessory flagellum short, two-articulated, last article minute.
Antenna 2 peduncular article 2 gland cone reaching
beyond end of peduncular article 3; article 4 subequal in
length to article 5, flagellum with nine articles. Lower lip
outer lobes with single pair of ducts, mandibular lobes ap-
ically acute. Mandible molar well developed, triturative,
with plumose seta; accessory setal row with four slender
blades; palp three-articulated; article 1 about as long as
broad, shorter than article 2; article 2 slightly longer than
article 3, with few long slender setae; article 3 longer than
article 1, weakly falcate, long (about 3.2 times as long as
broad), with comb of short robust setae along anterodis-
tal margin and three slender apical setae. Maxilla 1 inner
plate with two apical plumose setae; outer plate with sev-
en serrate robust setae; palp two-articulated, apical part
of palp article 2 with seven robust and two slender setae.
Maxilla 2 inner plate with five plumose setae and a few
slender setae. Maxilliped inner plate with two slender and
six plumose setae on apical part; outer plate bearing ten
medial/apical robust setae and six apical plumose setae.

Gnathopod 1 subchelate; coxa anterior margin slightly
concave, anterovelvetal corner produced and rounded,
ventral margin with few long setae; basis posterior margin
with four long setae; merus with posterodistal tooth; car-
pus about 1.5 times as long as broad, subequal in length to
propodus, heavily setose, with rows of long setae covering
the surface and posterior margin; propodus with few rows
of long marginal and superficial setae, palm acute, convex,
minutely serrate, defined by two posterodistal robust setae;
dactylus with one seta on anterior margin. Gnathopod 2
subchelate; coxa subrectangular, longer than broad, ventral
margin with some long setae; basis stout, posterior margin
with four long setae, anterodistal margin with group of five
long robust setae; merus anterovelvetal corner produced
and subquadrate; carpus posterovelvetal corner produced,
rounded and setose; propodus expanded, anterior and pos-
terior margin with rows and clusters respectively of long
slender setae; palm about half length of propodus, acute,
sculptured, with palm distal shelf subrectangular bearing
group of four robust setae, distal subquadrate tooth, medial
concave portion for reception of the dactylar tip, defined by
Figure 1. *Elasmopus yucalpeten* sp. n., holotype male, 6.6 mm, CYMX-1-EY; Yucalpeten harbor, Yucatan, Mexico. Scale bar for *H* represents 1 mm; scale bars for *G1* and *G2* represent 0.3 mm; scale bars for *AF* and *T* represent 0.1 mm.
Figure 2. *Elasmopus yucalpeten* sp. n., holotype male, 6.6 mm, CYMX-1-EY; Yucalpeten harbor, Yucatan, Mexico. Scale bars represent 0.1 mm.
proximal tooth; dactylus falcate, with one seta on anterior margin. Pereopod 3 similar to pereopod 4, except by the coxa subrectangular. Pereopod 4 coxa longer than broad, posterior margin concave, pterosternal margin produced and rounded, ventral margin with long setae; basis posterior margin with three long setae; propodus with a row of seven robust setae on posterior margin; dactylus short (about 0.5 times the propodus length), dactylar unguis simple. Pereopod 5–6 coxa broader than long, excavate ventrally, anterosternal lobe produced and rounded, with three pterosternal robust setae; basis posterior margin slightly expanded convex, weakly crenulate, with many long stiff slender setae; ischium, merus, carpus and propodus with many long, slender and robust setae along margins; dactylus short (about 0.3 times the propodus length), dactylar unguis simple. Pereopod 7 similar to pereopod 5–6, except by the coxa with ventral lobe produced and rounded, with one posterior robust seta.

Epimera 1–3 pterosternal margin notched with small subacute tooth; ventral margin with long slender and robust setae. Urosomite 1–3 smooth, without carina. Uropod 1 peduncle with two basofacial robust setae, five robust setae on each of the outer and inner margins, and two long robust setae distally; inner ramus about as long as peduncle. Uropod 2 peduncle with two and three robust setae on outer and inner margin, respectively; inner ramus longer than peduncle (about 1.2 times the peduncle length). Uropod 3 peduncle with two distal robust setae; inner ramus about as long as peduncle; outer ramus slightly longer than inner ramus with three clusters of long setae. Telson entire with posterior margin scalloped, two unequal stout robust setae and two or three plumose setae on each side.

Female (sexually dimorphic characters); based on paratype female, 6.2 mm (CYMX-2-EY). Gnathopod 1 subchelate; coxa ventral margin with short setae; basis medial surface with two long setae, posterior margin with seven long setae; carpus heavily setose, with long setae covering the surface and posterior margin; propodus with marginal and superficial setae, palm acute, convex, minutely serrate, with ten submarginal robust setae, defined by two posterodistal robust setae, followed by two submarginal robust setae; dactylus not reaching the end of palm, with one seta on anterior margin. Gnathopod 2 subchelate; coxa ventral margin with some long setae; basis posterior margin with five long setae; anterodistal margin with one long robust setae; carpus heavily setose, with long setae covering the posterior margin; propodus anterior and posterior margin with rows and clusters respectively of long slender setae, about twice length of carpus, palm acute, nearly straight, smooth, with 15 submarginal robust setae, defined by three posterodistal robust setae; dactylus falcate, not reaching the end of palm, with one seta on anterior margin. Pereopod 5–7 with few long stiff slender setae. Uropod 1 peduncle with one basofacial robust seta.

Variations; based on paratypes (CYMX-2-EY, CYMX-3-EY, ECOSUR0164). A group of six robust setae (instead of five) on the anterodistal margin of the basis of gnathopod 2 was observed in two of the ten male specimens. Male and female specimens were also examined for confirming the presence of two robust setae on peduncle of uropod 1, but there was not consistency; the rule was to present one basofacial robust seta.

Etymology. The species name is derived from the type locality of the new species. Yucalpeten in the Mayan language means “land of the deer”.

Distribution. So far only known from the type locality, Yucalpeten harbor, Yucatan, Mexico.

Habitat. Marine epibenthic, in shallow water (≤1 m) on rocks and wood dock with the brown algae Gracilaria sp.

Remarks. The presence of one or two basofacial robust setae on uropod 1 peduncle as a characteristic for species category identification may have been overlooked by earlier studies (Hughes and Lowry 2011); these same authors also have pointed out that, the presence of two basofacial robust setae on uropod 1 peduncle may be result of a newly-forming moult stage, such as the present case, where only the holotype male bears those spines. Elasmopus yucalpeten sp. n. is distinguished from the rest of the species in the genus by having the antenna 1 accessory flagellum two-articulated, mandibular palp article 3 weakly falcate, gnathopod 2 basis anterodistal margin with group of long robust setae, gnathopod 2 propodus palm with medial concave portion, uropod 1 peduncle with one or rarely two basofacial robust setae, and telson entire. An entire telson is only known in four other species of the genus, Elasmopus integer Myers, 1989, Elasmopus pseudinteger Appadoo & Myers, 2003, Elasmopus takamoitus Myers, 1986, and Elasmopus visakhapatnamensis Kanakadurga, Rao & Shyamasundari, 1981. Of these species, E. yucalpeten sp. n. is closely related to E. integer from Bora Bora, South Pacific, E. pseudinteger from Mauritius, Indian Ocean, and E. visakhapatnamensis from Visakhapatnam, Indian Ocean. It is distinguished from E. integer by the eyes ovate; mandibular palp article 3 weakly falcate; gnathopod 2 propodus palm with medial concave portion; pereopod 5–7 basis more setose; and epimera 2–3 pterosternal corner notched with small subacute tooth. It is distinguished from E. pseudinteger by the two-articulated accessory flagellum; the more setose mandibular palp article 2; the long slender setae along the anterior and posterior margin of the gnathopod 2 propodus; the entire, crenulate and more setose margin of the pereopod 5–6 basis; the margin evenly convex of the epimera 3 with the pterosternal corner notched and a small subacute tooth; the presence of one basofacial robust setae on the uropod 1 peduncle; and a telson with a scalloped posterior margin. Finally, it is distinguished from E. visakhapatnamensis by the following characteristics: mandibular palp article 3 weakly falcate; gnathopod 2 propodus palm with medial concave portion and proximal tooth; epimera 1–3 margin evenly convex with pterosternal corner notched, having a small subacute tooth; and uropod 3 outer ramus longer than inner ramus.
Figure 3. *Elasmopus yucalpeten* sp. n., holotype male, 6.6 mm, CYMX-1-EY; Yucalpeten harbor, Yucatan, Mexico. Scale bars represent 0.3 mm.
Figure 4. *Elasmopus yucalpeten* sp. n., holotype male, 6.6 mm, CYMX-1-EY; paratype female, 6.2 mm, CYMX-2-EY; Yucalpeten harbor, Yucatan, Mexico. Scale bars represent 0.3 mm.
Biogeographic comments

Up to now, nine species of Elasmopus (including the new species) have been recorded in the Gulf of Mexico (Table 1). At the horizontal axis, according to the regionalization of Felder et al. (2009) for the Gulf of Mexico basin, three of those species (E. levis, E. pocilimanus and E. rapax) have been widely reported in the Gulf basin regions, two species (E. balkomanus and E. pectenicrus) have been mostly reported in the northern regions, and the remaining four species are so far confined to the southeast region (E. cf. magnispinatus) or the northeast region (E. levis and E. yucalpeten sp. n.). At the vertical axis, according to the zonation by depth of Yáñez-Arancibia and Day (2004) for the Gulf of Mexico basin, all species have been reported on the coastal shallow (0–20 m) and only four species have been reported on the continental shelf (21–200 m). Furthermore, those species more widely distributed in the Gulf of Mexico also displayed a broad range of depth; in contrast, species with a constricted distribution displayed a narrow range of depth. Nevertheless, as LeCroy (2000) has pointed out for Elasmopus species narrowly distributed, further samplings may reveal that those species are actually more widespread than previously expected, for example, E. cf. magnispinatus has been reported only from the northeast region, but with a great number of records and a broad range of depth.

Regionally, the amphipod fauna in the Gulf of Mexico shows an affinity to the biogeographic provinces from the tropical western Atlantic (Carolinian and Caribbean) quoted by Neigel (2009) and Briggs and Bowen (2012). The Carolinian province corresponds to northern regions (NW and NE) representing a warm-temperate condition; whereas the Caribbean province corresponds to southern regions (SW and SE) representing a tropical condition. According to those provinces, the genus Elasmopus has species with tropical and temperate affinities. The tropical component is dominant with eight species, three of which are endemic so far for the Gulf of Mexico (Table 1). Globally, E. yucalpeten sp. n. is geographically related to four species which in turn are morphologically similar by a unique trait in the genus, an entire telson. Moreover, from those species, E. yucalpeten sp. n. is similar to three species of the rapax-group (E. integer, E. pseudinteger and E. visakhapatalemensis) by an anterodistal margin of gnathopod 2 basis with group of long robust setae and P5–7 basis having long setae, revealing possibly a separate complex of widely distributed species. The distribution of those species resembles the trans-Indo-Pacific-Caribbean tracks described by Myers and Lowry (2009) (Figure 5), which is explained by plate tectonic/sea-level changes during the Cretaceous and is represented by distribution of a number of amphipod taxa at the family category (e.g. Neomegamphopidae) and genus (e.g. Mallacoota, Shoemakerella). Therefore, with the similarity among species closely related to Elasmopus it was possible to recognize that biogeographic track, which proposes according to Myers (1991) and Myers and Lowry (2009) an ancient connection among seas and a current isolation by means of disjunct distributions of related taxa.

Table 1. Distribution data and biogeographic affinity of the Elasmopus species in regions of the Gulf of Mexico. Regions after Felder et al. (2009): SW, Southwest; NW, Northwest; NE, Northeast; SE, Southeast. Information based on Ortiz and Lalana (1994), LeCroy (2000), LeCroy et al. (2009), Vander and Krapp-Schickel (2012), and Paz-Ríos et al. (2013).

| Species                  | SW  | NW | NE | SE | Depth (m) | Carolinian | Caribbean |
|-------------------------|-----|----|----|----|-----------|------------|-----------|
| E. balkomanus           | ●   | ●  | ●  |    | 1–3       | ●          | ●         |
| E. lemairei             | ●   | ●  | ●  | ●  | <1–3      | ●          | ●         |
| E. levis                | ●   | ●  | ●  | ●  | 0–18      | ●          | ●         |
| E. pectenicrus          | ●   | ●  | ●  | ●  | 0–50      | ●          | ●         |
| E. pocilimanus          | ●   | ●  | ●  | ●  | 0–30      | ●          | ●         |
| E. rapax                | ●   | ●  | ●  | ●  | 1–50      | ●          | ●         |
| E. thomasi              | ●   | ●  | ●  | ●  | <1–3      | ●          | ●         |
| E. cf. magnispinatus    | ●   | ●  | ●  | ●  | 6–55      | ●          | ●         |
| E. yucalpeten sp. n.    | ●   | ●  | ●  | ●  | ≤1         | ●          | ●         |
Acknowledgments

We thank Maria Teresa Herrera-Dorantes (Laboratorio de Bentos, Cinvestav) for her support during the field sampling, as well as to Luis F. Carrera Parra and Norma E. González Vallejo (ECOSUR, Chetumal) for the deposition of a part of the type material in the reference collection of ECOSUR. Special thanks are also due to Rachael A. Peart (Australian Museum, Sydney) for reading the manuscript and for her valuable comments/suggestions. The authors thank two reviewers who kindly helped to improve the manuscript.

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Natural history museums as repositories of endangered diversity: the case of the United States Unionida in the Museo di Zoologia dell’Università di Bologna

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Abstract

The importance of natural history museums is often underappreciated, but they provide society with a number of services. Among these, they are a fundamental tool for assessing extinction rates and range contractions, or the only way to access species extinct in historical times. In this perspective, we describe here the collection of Unionida of the Museo di Zoologia dell’Università di Bologna, containing one extinct (*Epioblasma haysiana*) and nine threatened species, plus another 47 species. The collection was built in the mid-19th century and potentially provides baseline information for specialists. In the fragmented natural history museum system of Italy, this might be just the tip of the iceberg of a significant and important amount of material collected in the 19th and early-20th century.

Key Words

Unionidae
Margaritiferidae
extinct species
museums
natural history collections

Introduction

Natural history museums provide society with a number of indispensable, although often underappreciated, services in the fields of homeland security, public health and safety, agriculture, monitoring of environmental change, traditional taxonomy and systematics (Suarez and Tsutsui 2004). They are also indispensable for the study of the state and trends of biodiversity, providing baseline information useful to assess change at the genetic, species, community and landscape level. They are sometimes the only repositories of specimens of taxa which have gone extinct in historical times, and allow studies on endangered taxa avoiding new captures.

The ‘Museo di Zoologia dell’Università di Bologna’ is no exception. In its present form, it dates back to the 1930s, but it actually contains specimens dating back to the 16th century (collected by Ulisse Aldrovandi, 1522-1605). It was then enriched by the collections of F. Cospi (1609-1686) and L.F. Marsigli (1658-1730), and further developed during the 19th century, mainly due to the work of its directors C. Ranzani (1775-1841) and G.G. Bianconi (1809-1878), and 20th century, due to the commitment of A. Ghigi (1875-1970). The value of the museum as repository of specimens of extinct or endangered species has been already recognized for vertebrates; for example it contains a head of a great auk, *Pinguinus impennis* (Linnaeus, 1758), which became extinct in the mid-19th century. However, no recognition was ever given to the value of its invertebrate collections, of which here we present the North-American Unionida.

The Unionida is a diverse order of bivalves with ca. 840 species worldwide. The Nearctic (especially the SE United States) has the highest concentration of Unionida diversity in the world, comprising ca. 300 species alone (Graf and Cummings 2007, Bogan 2008). However, this richness has been threatened by the construction of dams, pollution and sediment toxicity, wetland drainage and channelization, sedimentation and siltation resulting from poor agricultural and silvicultural practices, highway and bridge
construction, interbasin transfer schemes, habitat loss through dredging, and other land-use activities (Lyeard et al. 2004). At present, 235 species have been assessed, of which 27 (11%) are considered extinct, 50 (21%) critically endangered, 31 (13%) endangered and 11 (5%) vulnerable (IUCN Red List, last accessed July 2013).

Our work recovered the collection of Unionida in the Museo di Zoologia dell’Università di Bologna, Italy, a collection dating back to the 19th century. This collection is likely to be only the tip of the iceberg among many other collections, hidden in the Italian fragmented natural history museum system. Our aims are therefore to highlight the value of historic natural history collections as repositories of specimens of extinct or endangered species. In a time of increased awareness of global biological changes, museums are valuable reservoirs of baseline information. Moreover, we wish to bring to the attention of the international scientific community the hidden treasures in the Italian museums, and foster research on their material.

Methods

We recovered the collection of North American Unionida, cleaned all specimens, labels and original boxes, and transferred them into zip-lock plastic bags along with all original labels. Also the original boxes, likely to belong to early 20th century were preserved. The most interesting lots were photographed. Identification was checked, nomenclature updated following the MUSSELp database (Graf and Cummings 2013), which proved particularly useful in tackling old names, and the conservation status of each species was recorded on the basis of published assessments (IUCN Red List, U.S. Fish and Wildlife Service). Samples were databased, and research into the biography of the main contributors to the collection performed. The collection also contains several lots from Europe, as well as samples from South America, Africa and Asia, which were cleaned, but not analysed in detail.

Results

Eighty-six specimens of North-American Unionida are preserved in the Museum (Table 1 gives a list) in 76 lots, representing two families (Unionidae and Margaritiferidae) and 57 species. The condition of specimens is generally very good, with most valves being paired, and only a few having cracks or other defects. The collection comprises further 34 lots (104 specimens and 22 valves) from Europe, 21 lots (31 specimens and two valves) from the Central and South America, three lots (three specimens) from sub-Saharan Africa, and two lots (two specimens) from New Zealand, which will not be further commented upon here.

Of these 57 species, 10 (17.5%) are currently considered extinct or threatened by IUCN. In particular, one is considered extinct (Epioblasma haysiana (Lea, 1834), Figure 1 E-F), five critically endangered, two endangered and two vulnerable. The U.S. Fish and Wildlife Service lists as “endangered” further three species not assessed by IUCN: Epioblasma oblitiata (Rafinesque, 1820), E. torulosa rangiana (Lea, 1838) and E. triquetra (Rafinesque, 1820).

Most of the lots were received from M.E. Moricand (1779-1854) (Fig. 2 A-B), a Swiss collector, in the mid-1800s. His collection was particularly rich, especially of land shells: his son M.J. Moricand catalogued his collection in 1859 censuring 5,950 species and about 25,000 specimens (Cailliez, 1983). A few more lots from the Mississippi River are dated 1863 and belonged to the Capellini collection (Fig. 2 C). Giovanni Capellini (1833-1922) was a professor of geology at the University of Bologna, and travelled in 1863 to the United States (Vai, 2002).

Discussion

Museum collections are increasingly becoming the only source of information on extinction rates and range contractions, or the only way to access species extinct in historical times (Allmon 1994). The case study here reported shows that the effort to recover an historical 19th century collection allowed the recognition of an extinct species and of several endangered species, belonging to one of the most imperiled groups of molluscs worldwide. The specimens themselves, and the data accompanying them, provide researchers with a historic record of where extinct and endangered species once lived. Notwithstanding natural history collections are then a vital resource for conservation research, the recognition of their importance is often lacking.

Even when the value of natural history collections as sources of long-term or past datasets is recognized (Lister et al. 2011), locating material of interest is often difficult due to the fragmentation of the museum system in some countries, the lack of computerization of collection data, or the lack of availability to the public (e.g. via internet) (O’Connell et al. 2004). The Museo di Zoologia dell’Università di Bologna does not have permanent personnel, and all curatorial work is carried out by university researchers and students in the framework of their research assignments or on voluntary basis. The malacological collection is partly databased, but the database is not readily available to the public. Publishing the results of this work will hopefully contribute to arise consciousness on the value of natural history collections, and start spreading information to the scientific community on the Italian invertebrate historical collections. Indeed, the Italian museum system had its golden years in the second half of the 19th century and early 20th century (see for a narrative on vertebrate collections Gippoliti (2005)). Therefore, further collections of interest can be expected when digging into the old cabinets of the Italian museums.
Table 1. List of North-American Unionida preserved at the Museo di Zoologia dell’Università di Bologna, Italy. Abbreviations: R.: River; IUCN: International Union for the Conservation of Nature; USFWS: United States Fish and Wildlife Service; IUCN categories: DD Data Deficient, LC Least Concern, NT Near Threatened, VU Vulnerable, EN Endangered, CR Critically Endangered, EX Extinct.

| Species | Locality | Number of specimens | Source | Conservation status |
|---------|----------|---------------------|--------|---------------------|
| Actinonaias ligamentina (Lamarck, 1819) | Ohio R. | 2 lots, three specimens | M.J. Moricand | LC (IUCN) |
| Actinonaias cfr. ligamentina (Lamarck, 1819) | No locality | 2 lots, 2 specimens | Not known | Not evaluated |
| Alasmidonta heterodon (Lea, 1829) | Connecticut R. | 1 lot, 1 specimen and 1 valve | M.J. Moricand | VU (IUCN) |
| Alasmidonta marginata Say, 1818 | Wabash R. | 1 lot, 1 specimen | M.J. Moricand | DD (IUCN) |
| Alasmidonta undulata (Say, 1817) | Delaware R. | 1 lot, 2 specimens | M.J. Moricand | LC (IUCN) |
| Alasmidonta varicosa (Lamarck, 1819) | Delaware R. | 1 lot, 1 specimen | M.J. Moricand | DD (IUCN) |
| Ambplema plicata (Say, 1817) | Ohio R. | 2 lots, 2 specimens | M.J. Moricand | LC (IUCN) |
| Amphinaias nodulata (Rafinesque, 1820) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | LC (IUCN) |
| Amphinaias pustulosa (Lea, 1831) | Ohio R.; Mississippi R. | 2 lots, 2 specimens | M.J. Moricand; G. Capellini (1863) | LC (IUCN) |
| Cyprogenia stegaria (Rafinesque, 1820) | Ohio R. | 2 lots, 2 specimens | M.J. Moricand (1844) | CR (IUCN) |
| Dromus dromas (Lea, 1834) (Fig. 1 C-D) | Cumberland R. | 1 lot, 1 specimen | M.J. Moricand | CR (IUCN), Endangered (USFWS) |
| Ellipsaria lineolata (Rafinesque, 1820) | Ohio R.; Mississippi R. | 2 lots, 2 specimens | M.J. Moricand; G. Capellini (1863) | NT (IUCN) |
| Elliptio complanata (Lightfoot, 1786) | Delaware R. | 1 lot, 1 specimen | M.J. Moricand | LC (IUCN) |
| Elliptio congareae (Lea, 1831) | Altamaha R. | 1 lot, 1 specimen | M.J. Moricand | NT (IUCN) |
| Elliptio crassidens (Lamarck, 1819) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | LC (IUCN) |
| Elliptio cylindracea (Frierson, 1927) | Altamaha R. | 1 lot, 1 specimen | M.J. Moricand | DD (IUCN) |
| Elliptio dilatata (Rafinesque, 1820) | Ohio R.; Illinois | 2 lots, 2 specimens | M.J. Moricand | LC (IUCN) |
| Elliptio fisheriana (Lea, 1838) | Maryland | 1 lot, 1 specimen | M.J. Moricand | LC (IUCN) |
| Elliptio hopotensensis (Lea, 1838) | Altamaha R. | 2 lots, 2 specimens | M.J. Moricand | LC (IUCN) |
| Elliptio lanceolata (Lea, 1828) | Tennessee | 1 lot, 1 specimen | M.J. Moricand | NT (IUCN) |
| Elliptio shepardiana (Lea, 1834) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | NT (IUCN) |
| Epiblasma haysiana (Lea, 1834) (Fig. 1 E-F) | Cumberland R. | 1 lot, 1 specimen | M.J. Moricand | EX (IUCN) |
| Epiblasma obliquata (Rafinesque, 1820) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | Not evaluated (IUCN), Endangered (USFWS) |
| Epiblasma torulosa (Rafinesque, 1820) (Fig. 1 G-H) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | CR (IUCN), Endangered (USFWS) |
| Epiblasma torulosa rangiana (I. Lea, 1838) | Ohio R. | 1 lot, 2 specimens | M.J. Moricand | Not evaluated (IUCN), Endangered (USFWS) |
| Epiblasma triquetra (Rafinesque, 1820) | Ohio R. | 1 lot, 1 specimen | M.J. Moricand | Not evaluated (IUCN), Endangered (USFWS) |
| Fusconaia flava (Rafinesque, 1820) | Ohio R.; Mississippi R. | 2 lots, 4 specimens | M.J. Moricand; G. Capellini (1863) | LC (IUCN) |
| Lampsis cariosa (Say, 1817) (Fig. 1 I-J) | Delaware R. | 2 lots, 2 specimens | M.J. Moricand (1844) | EN (IUCN) |
| Lampsis hydiana (Lea, 1838) | Louisiana | 1 lot, 1 specimen | M.J. Moricand | LC (IUCN) |
| Species                          | Locality                   | Number of specimens | Source                          | Conservation status |
|---------------------------------|----------------------------|---------------------|---------------------------------|---------------------|
| *Lampsilis siliquoidea* (Barnes, 1823) | Ohio                       | 2 lots, 3 specimens | M.J. Moricand                  | LC (IUCN)           |
| *Lampsilis* sp.                 |                            | 1 lot, 1 specimen   |                                 |                     |
| *Lampsilis teres* (Rafinesque, 1820)  | Mississippi R., Louisiana  | 2 lots, 2 specimens | G. Capellini (1863); M.J. Moricand | LC (IUCN)           |
| *Lasmigona compressa* (Lea, 1829) | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | LC (IUCN)           |
| *Lasmigona costata* (Rafinesque, 1820) | Ohio                       | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN)           |
| *Leptodea ochracea* (Say, 1817)  | Delaware R.; North America | 2 lots, 3 specimens | M.J. Moricand; Champlain        | NT (IUCN)           |
| *Ligumia nasuta* (Say, 1817)     | Mississippi R.             | 1 lot, 1 specimen   | G. Capellini (1863)             | NT (IUCN)           |
| *Ligumia recta* (Lamarck, 1819)  | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | LC (IUCN)           |
| *Medionidus conradicus* (Lea, 1834) | Holston R. in Tennessee    | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN)           |
| *Obliquaria reflexa* (Rafinesque, 1820) | Ohio R.; Mississippi       | 2 lots, 4 specimens | M.J. Moricand; G. Capellini (1863) | Not evaluated       |
| *Obovaria olivaria* (Rafinesque, 1820) | Mississippi R.             | 1 lot, 1 specimen   | G. Capellini (1863)             | LC (IUCN)           |
| *Obovaria subrotunda* (Rafinesque, 1820) | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN)           |
| *Pleurobema clava* (Lamarck, 1819) | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | CR (IUCN), Endangered (USFWS) |
| *Pleurobema oxiiforme* (Conrad, 1834) | Haledon R.                 | 2 lots, 3 specimens | M.J. Moricand                  | VU (IUCN)           |
| *Pleurobema rubrum* (Rafinesque, 1820) | North America              | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN)           |
| *Pleurobema sintoxia* (Rafinesque, 1820) | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | LC (IUCN)           |
| *Potamilus alatus* (Say, 1817)   | 2 lots without label; third: Mississippi R. | 3 lots, 3 specimens | 2 lots without label; third: G. Capellini (1863) | LC (IUCN)           |
| *Pyganodon gibbosa* (Say, 1824)  | Altamaha R.                | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN)           |
| *Quadrula cylindrica* (Say, 1817) | Cumberland R.              | 1 lot, 1 specimen   | M.J. Moricand                  | NT (IUCN), Threatened (USFWS) |
| *Quadrula quadula* (Rafinesque, 1820) | Mississippi R.            | 1 lot, 1 specimen   | G. Capellini (1863)             | LC (IUCN)           |
| *Quadrula metanevra* (Rafinesque, 1820) (Fig. 3 C-D) | Ohio                      | 1 lot, 1 specimen   | M.J. Moricand                  | CR (IUCN)           |
| *Quadrula verrucosa* (Rafinesque, 1820) | Ohio R.                   | 1 lot, 1 specimen   | M.J. Moricand                  | Not evaluated       |
| *Taxolasma lividum* (Rafinesque, 1831) | Ohio R.                   | 1 lot, 1 specimen   | M.J. Moricand                  | Not evaluated       |
| *Taxolasma parvum* (Barnes, 1823) | Ohio R.                    | 1 lot, 1 specimen   | M.J. Moricand                  | LC (IUCN)           |
| *Truncilla donaciformis* (Lea, 1828) | North America; Ohio       | 2 lots, 1 specimen and 1 valve | M.J. Moricand                  | LC (IUCN)           |
| *Utterbackia imbecillis* (Say, 1829) | Ohio R.                   | 1 lot, 3 specimens  | M.J. Moricand                  | LC (IUCN)           |
| Unidentified                    |                            | 2 lots, 1 complete specimen and 1 valve | –                             | –                  |
| *Margaritifera margaritifera* (Linnaeus, 1758 (Fig. 3 E-F) | Small streams in Pennsylvania | 1 lot, 1 specimen | M.J. Moricand                  | EN (IUCN)           |
Figure 1. North-American Unionida of conservation concern in the Museo di Zoologia dell’Università di Bologna, Italy. **A-B.** Cyprogenia stegaria (Rafinesque, 1820), Critically Endangered (IUCN), Cumberland River, length 40 mm; **C-D.** Dromus dromas (Lea, 1834), Critically Endangered (IUCN), Endangered (USFWS), Cumberland River, length 64 mm; **E-F.** Epioblasma haysiana (Lea, 1834), Extinct (IUCN), Cumberland River, length 41 mm; **G-H.** Epioblasma torulosa (Rafinesque, 1820), Critically endangered (IUCN), Endangered (USFWS), Ohio River, length 56 mm; **I-J.** Lampsilis cariosa (Say, 1817), Endangered (IUCN), Delaware River, length 76 mm. Scale bar 1 cm.
Figure 2. Examples of original labels of North-American Unionida in the Museo di Zoologia dell’Università di Bologna, Italy. A. M.E. Moricand’s label, in Latin. B. Labels accompanying M.E. Moricand’s specimens in English. C. G. Capellini’s label.

Figure 3. North-American Unionida of conservation concern in the Museo di Zoologia dell’Università di Bologna, Italy. A-B. *Pleuronoba clava* (Lamarck, 1819), Critically Endangered (IUCN), Endangered (USFWS), Ohio River, length 50 mm; C-D. *Quadrula metanevra* (Rafinesque, 1820), Critically Endangered (IUCN), Ohio River, length 59 mm; E-F. *Margaritifera margaritifera* Linnaeus, 1758, Endangered (IUCN), small streams in Pennsylvania, length 123 mm. Scale bar 1 cm.
Acknowledgements

We thank Arthur E. Bogan for his patient and long-lasting support to our work.

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The rediscovery of Perret’s toad, *Amietophrynus perreti* (Schiøtz, 1963) after more than 40 years, with comments on the species’ phylogenetic placement and conservation status

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Abstract

Perret’s toad, *Amietophrynus perreti*, has not been seen since 1970 and thus believed to be lost. We searched for the species 50 years after its original description and successfully recorded its continued presence at the type locality, where it seems to maintain a viable population. We failed however, to record the species at suitable sites elsewhere and *A. perreti* could thus indeed be a micro-endemic species, specialized and restricted to the granite inselbergs of the Idanre Hills, Nigeria. We recorded and discuss potential threats and suggest keeping the toad’s current conservation status as ‘Vulnerable’. We investigated the systematic status of Perret’s toad using a mitochondrial fragment of the 16S rRNA gene and could confirm that it is a member of the genus *Amietophrynus* despite its aberrant larval biology, different to the rest of the genus. In spite of this biological difference, *A. perreti* is not a phylogenetically isolated lineage, but is nested within a clade of western African *Amietophrynus* species, such as *A. maculatus*, *A. regularis*, *A. latifrons* and *A. togoensis* and is sister to the widespread and savannah dwelling *A. maculatus*.

Key Words

Amphibia
Anura
Bufonidae
conservation
ecology
inselberg
morphology
Nigeria
phylogeny
systematics
West Africa

Introduction

In 1963 Arne Schiøtz described a new toad from inselbergs, isolated old granite outcrops, in the Idanre Hills “near Idanre Resthouse, Ondo Province”, southwestern Nigeria (Schiøtz 1963). This new species, *Bufo perreti*, dedicated to the Swiss herpetologist Jean-Luc Perret, is morphologically similar to the common African toad species *Amietophrynus maculatus* (Hallowell, 1854) and *A. regularis* (Reuss, 1833). However, among various morphological details the new species differed by a fundamentally aberrant breeding biology. Whereas all other known *Amietophrynus* species deposit strings with numerous small eggs in water (Liedtke et al. 2014), from which exotrophic and aquatic tadpoles with very similar morphologies are hatching (compare Channing et al. 2012), *A. perreti* has semi-terrestrial egg deposition (Tandy and Keith 1972) and very slender shaped, semi-
terrestrial tadpoles, developing in shallow water-films on wet, sometimes vertical rocks (Schiøtz 1963; Fig. 1).

Since its description the species has rarely been mentioned in the literature. Based on osteological characters (Martin 1972) and acoustic criteria (Tandy and Keith 1972) it was placed in a monotypic group within the genus *Bufo*. Mills Tandy studied the three toads *A. perreti*, *A. maculatus* and *A. regularis* in 1964 and 1965, occurring in sympathy at the *A. perreti* type locality, and observed that they differ in size, advertisement call parameters, reproductive season and biology, as well as in microhabitat selection (Tandy and Keith 1972). Based on karyology (2n= 20; Bogart 1968), but without genetic data, Frost et al. (2006) transferred *B. perreti* into their new genus *Amietophrynus*, and Channing et al. (2012) redescribed the tadpoles. Schiøtz and Tandy (2004) and Stuart et al. (2008) give the conservation status of the species as 'Vulnerable'. However, only Mills Tandy seems to have collected new data from the field (Tandy and Keith 1972) and until now, the species is still only known from its type locality. On the webpage of the Amphibian Survival Alliance (ASA 2014) *Amietophrynus perreti* is listed as being lost, the last observation dating to 1970. We do not know from which data the later date stems. In 2013, 50 years after its original description, we decided to search for the species. Our aims were fourfold: i) document if the species still exists at the type locality, ii) collect data on the population status, iii) search in neighbouring areas for further populations, and iv) investigate the systematic position of this species.

**Material and methods**

All field work was carried out by two persons (ABO and a field assistant) in the Idanre Hills, Ondo Province, south-western Nigeria (approximately 7°06′N, 5°06′E; see Appendix). The region was visited three times; the first visit from 10-12 October 2013 was only used to get familiar with the topography and to select a suitable field assistant. The second visit took place at the beginning of the dry season (26-28 October 2013). During three days and two nights we applied visual and acoustic encounter surveys (compare Rödel and Ernst 2004) at three sites (see below). Day surveys were conducted at all sites while night surveys were conducted at sites A and C only. The third visit comprised one day and one night survey at sites A and B, respectively, and took place from 13–14 March 2014, the ending of the dry season (Table 1).

Site A (7°06′37.3″N, 5°06′24.1″E) comprised approximately 2 ha situated between two huge rocks, only about 50 m from the town of Idanre (Fig. 2a, c). Water flowed in two valleys between these rocks. The vegetation consisted mainly of shrubs and small trees. Site B (7°06′14.2″N, 5°04′41.3″E), covering about 1.5 ha, was approximately 250 m height on the rocks. This area was relatively flat with patches of vegetation, shrubs and trees, growing on the rocks. Water was flowing down from higher locations, forming rock-pools in some depressions (Fig. 2b). Site C (7°06′32.9″N, 5°04′28.0″E) was part of a cocoa farm adjacent to a large inselberg, including a creek flowing through the farm (Fig. 2e). The creek had a rocky bottom and was 6-11 cm deep. Along the water course, there were few grasses and shrubs.

In addition, we surveyed two further sites with similar habitats (rocky inselbergs). Ikere was about 28 km from Idanre. Two areas were surveyed here on 3-4 November 2013 and 16 March 2014. Site 1 comprised giant rocks and was surveyed for 6 and 4 hours per day and night, respectively (3 day and 2 night surveys, each 2 hours length). With the same sampling effort we investigated site 2, a cocoa plantation near rocks. The second area was situated about 6 km from Akure and was searched on 6 November 2013 for 3.5 hours during the day and night, respectively. A map of all surveyed sites is provided in Figure 3.

All amphibians encountered were recorded. A few vouchers were collected to ascertain species determination. They were euthanized in a chlorobutanol solution and preserved in 75% ethanol. Vouchers are deposited at the zoological collection of A.B. Onadeko at the University of Lagos (no accession numbers) and at the Museum für Naturkunde Berlin (see Appendix for ZMB and GenBank...
Table 1. Time period and time span spent searching for *Amietophrynus perreti* per site in the Idanre Hills. In parenthesis total search time per site is given in person-hours.

| Date            | 26-28 October 2013 | 13 March 2014 | 14 March 2014 |
|-----------------|--------------------|---------------|---------------|
|                 | Day | night       | day | night | day | night |
| A (17 p-h)      | 14:00-16:00 | 21:00-23:00 | 15:00-17:30 | 21:00-23:00 |
| B (16 p-h)      | 08:00-10:00 | –           | 08:00-11:00 | 18:00-21:00 |
| C (6 p-h)       | 11:00-13:00 | 19:00-20:00 | –           | –          |

accession numbers). Muscle samples of some toads were investigated to test for their systematic placement. Unfortunately only two samples could be genotyped, probably due to impure ethanol used for tissue storage. For the molecular procedures applied compare Rödel et al. (2012). Sequences, ca. 575 base pairs of the mitochondrial 16S gene,
Figure 3. Sites around Idanre Hills surveyed for *Amietophrynus perreti*; green circles: toads recorded, red crosses: toads not found.

Figure 4. A specimen of the rediscovered *Amietophrynus perreti* from the type locality, the Idanre Hills, south-western Nigeria.

were compared to GenBank data as well as sequences for additional *Amietophrynus* species of interest (see results). Sequences were aligned using MAFFT v7.017 (Katoh and Standley 2013) and a Bayesian phylogenetic inference was carried out using MrBayes version 3.2.2 (Ronquist et al. 2012). Four independent runs were executed to assess convergence, each for 3 million generations, sampling every 1000 generations. Four chains per run were allowed to sample across the entire general time reversible substitution model space and the first 10% of each run were discarded as burn-in before generating a 50% majority-rule consensus tree from the posterior tree samples. Uncorrected 16S r-distances of *A. perreti* spp. *Amietophrynus* and included *Amietophrynus* spp. were calculated using PAUP* 4b10 (Swofford 2003).

Results

**Occurrence, abundance and threats.** We rediscovered *Amietophrynus perreti* and observed them in sympatry with *A. regularis*, *Phrynobatrachus* spp. and *Arthroleptis* spp. at the study sites of the Idanre Hills inselbergs. We did not observe any *A. maculatus*, known to occur here as well (Tandy and Keith 1972). Occasionally we spotted *A. regularis* in close vicinity to *A. perreti*, but then usually at the rock bases. *A. regularis* were particularly dominant around the town of Idanre, where *A. perreti* was lacking. Puddle frogs, *Phrynobatrachus*, were observed on the rocks, in vegetation around water-filled rock-pools. *Arthroleptis* were abundant in the cocoa farm and in tertiary vegetation but absent from the rocks. In total we observed 111 *A. regularis*, 49 *Phrynobatrachus* spp. and 98 *Arthroleptis* spp. during 39 person-hours of searching.

*Amietophrynus perreti* (Fig. 4) were observed during day and night, mainly on higher parts of the rocks. They were encountered on the hard rock surfaces and among low herbal vegetation on sandy soil between the rocks. During October 2013 we observed a total of 31 *A. perreti*, 16 during the day (12 person-hours search time, Table 1) and 15 during the night (6 p-h). In March 2014 we recorded a total of 12 *A. perreti*, 5 during day (11 p-h) and 7 (10 p-h) during night time (Table 2). Compared to the other anuran taxa, *A. perreti* had the lowest abundances.

We observed two potential threats to the survival of the toads. Most importantly some areas around the sites are being cleared for cocoa plantations, the major cash crop in the region. At higher places on the rocks some crops such as pepper, corn and banana are planted. Another cash making venture is the intense cutting of trees that grow among the rocks. We could not observe *A. perreti* in two other localities, namely Akure, 6 km and Ikere, about 28 km from Idanre. Both had similar habitats and topography compared to the *A. perreti* type locality.

**Morphology.** We examined three male and four female *A. perreti* vouchers (see Appendix). The males’ snout-vent-lengths (SVL: 39.4-40.2 mm) were within the range Schiøtz (1963) and Tandy and Keith (1972) provided for their specimens. None of the presumed male toads exhibited nuptial pads (dry season and hence no reproductive season). The females (55.3-64.4 mm SVL) slightly exceeded the sizes of the known females (Schiøtz 1963, Tandy and Keith 1972). Small differences to the original description concern the size of the metatarsal tubercles, being quite big and clearly distinct (larger in females than in males), and the presence of small, very flat and indistinct warts on the top of the head (no warts according to original description). All toads had comparatively flat heads, slightly pointed in lateral view (rounded in *A. regularis* and *A. maculatus*), almost no webbing between toes (distinct webbing between toes in *A. regularis* and *A. maculatus*), no tarsal fold (present in *A. regularis* and *A. maculatus*), and a white throat in males (dark in *A. regularis* and *A. maculatus*). In the preserved vouchers (some of them very flattened after transportation) the parotid glands ranged from less distinct to slightly more pronounced than in *A. maculatus*, but they were never as huge and smooth as in *A. regularis*. All our vouchers had more or less the same dorsal pattern as the holotype (compare Fig. 4). In life they have the olive (to yellowish) tinge Schiøtz (1963) mentioned in his description. All
preserved animals had a much clearer and more contrasting pattern than *A. regularis* and *A. maculatus*.

**Generic assignment.** Tandy and Keith (1972) placed *A. perreti* in its own, monotypic group, although having the same number of chromosomes as the *A. regularis-maculatus-latifrons* complex (2n= 20, Bogart 1968). A crossing of a female *A. maculatus* with a male *A. perreti* produced some embryos to the larval stage, however none of them reached metamorphosis (Blair 1972) [such crossing worked to gastrula or even larval stage even with American species nowadays placed in different genera, namely *Anaxyrus boreas*, see Blair 1972].

Our Bayesian phylogenetic inference based on 16S sequence data placed *A. perreti* as sister to *A. maculatus* (Fig. 5), with a pair-wise base difference of 7.3% (GenBank: GU183858). Pair-wise differences with other African *Amietophrynus* were: 7.86% in *A. mauritanicus* (GB: FJ882826), 9.25% in *A. channingii* (HQ882843), 9.29% in *A. regularis* (GB: AF220889), 9.60% in *A. gutturalis* (GB: GQ183567), 9.64% in *A. camerunensis* (GB: DQ158439), 9.64% in *A. poweri* (GB: DQ158482), 9.66% in *A. superciliaris* (GB: HQ882848), 9.82% in *A. kisoloensis* (GB: DQ158464), 9.87% in *A. rangeri* (GB: AF220868), 10.26% in *A. pardalis* (GB: AF220897), 10.43% in *A. pantherinus* (GB: AF220896), 10.47% in *A. garmani* (GB: AF220885), 11.19% in *A. braunii* (GB: DQ158437), 11.23% in *A. lemaiirii* (GB: AF220895), 11.66% in *A. latifrons* (GB: KF665409), 12.15% in *A. xeros* (GB: DQ158499), 12.29% in *A. tuberosus* (GB: DQ283362), and 12.42% in *A. togoensis* (GB: KF665445). There were no base pair differences between the two sequenced *A. perreti* specimens.

**Discussion**

We succeeded in rediscovering *Amietophrynus perreti* at its type locality, 50 years after its original
description, and 43 years after the last observation, if the date of 1970 is correct (ASA 2014). A welcome by-product of our investigations was that we could confirm the morphological description of the species by Schiøtz (1963), and more importantly, confirm that it is indeed a member of the genus Amietophrynus. It was however, surprising, and in contrast to the osteological results of Martin (1972) and the biological and acoustical analyses of Tandy and Keith (1972), that A. perreti has no isolated phylogenetic position within the genus, but seems to be most closely related to the savannah dwelling A. maculatus (see Rödel 2000 for details of the biology of this species). The 16S sequence data did not sufficiently resolve a number of nodes in the phylogeny, however, the Amietophrynus crown group was well supported, as was the A. perreti-maculatus-regularis-latifrons-togoensis clade.

The long time gap since the previous observations and the rediscovery of the toad was not due to particular rarity of the species but can be explained by the fact that nobody else searched for the species. We found the toad being locally quite abundant, although our search time at the beginning and towards the end of the dry season certainly was suboptimal. According to Tandy and Keith (1972) A. perreti reproduces during the core rainy season.

Due to their age and peculiar edaphic and climatic conditions, inselbergs are very special habitats with often unique plant and animal communities (Porembski and Barthlott 2000). Unfortunately, so far they have attracted almost exclusively the attention of geologists and botanists. We only know a few studies either focusing entirely (e.g. McLachlan and Cantrell 1980, McLachlan 1981, Osborne and McLachlan 1985, Köhler and Böhme 1996, Schorr 2003) or partly on amphibians on inselbergs (Rödel 1998). As far as we know, A. perreti is the only African anuran species depending exclusively on this habitat type. Schiøtz and Tandy (2004) speculate that A. perreti may occur in other areas of western Nigeria, offering similar habitats, but we did not record the species in two similar areas in closer vicinity of the type locality, nor was the species recorded by Onadeko and Rödel (2009) from other sites in south-western Nigeria. It thus seems possible that A. perreti is indeed an endemic species of the Idanre Hills.

During the current survey we observed no tadpoles of A. perreti. However, the tadpoles require a constant water film on the rocky surfaces where they graze on algae (Fig. 1), most likely in a situation like figured in Figure 2b. During the dry season, although occasional rains occur, such habitats do not exist. A reason for the absence of A. perreti on other inselbergs might be that the existence of this species, with its very special reproductive biology, requires an environment with high humidity persisting for prolonged periods and some water storing vegetation. Only then it seems possible that a constant water film on the rocks is maintained and provides the tadpoles of A. perreti with enough time to finish metamorphosis. The tadpoles seem to actively avoid deeper water and instantly, when forced into water, climb out again (Schiøtz 1963), showing a behavior comparable to some Petrophysides tadpoles (Barej et al. 2010). It thus seems that they will not make use of deeper rock-pools (Fig. 2e) and ultimately depend on the wet rock surfaces (Fig. 1).

We know from other inselbergs in the forest zone of Ivory Coast, that these provide a much hotter and dryer environment compared to the surroundings and thus are home to true savannah species, even within a rain-forest matrix (Schorr 2003). The fact that true forest in south-western Nigeria disappeared almost completely (see Onadeko and Rödel 2009), may explain that A. perreti simply cannot survive on other nearby inselbergs because the overall climate is too hot and dry. Already Schiøtz (1963) had failed to record the species in nearby inselbergs, surrounded by savannah vegetation. It is also possible that the mere dimensions of the inselberg landscape near Idanre are simply unequaled elsewhere.

According to the original description (Schiøtz 1963) A. perreti is abundant within its habitat. Arne Schiøtz collected 16 adults in July and August, 10 semi-adults and juveniles in January, July and August and 74 tadpoles in July and August 1961. On our surveys we likewise encountered the species frequently, although probably less frequent than Schiøtz (1963). We guess that the toads are not only reproducing during the rainy season (Tandy and Keith 1972), but that this is also the peak activity period and that the Idanre Hills population is still in good shape. The population thus seems viable, but we do not entirely agree with the judgment of Schiøtz and Tandy (2004) that the species does not face “any serious threats at present” because the “rocky habitat is largely inaccessible and useless to humans”. Schiøtz (1963) reported that he found all adults at night on bare rocks. He heard males calling in July 1961 during daytime, the voices coming from “very small patches of grass and low vegetation on the rocks, never from the wooded area” (Schiøtz 1963). Tandy and Keith (1972) report similar observations. It thus seems that the toads, at least during the rainy season, do not need forested areas. However, we believe that the current clearing of larger trees from the area, and the use of smaller patches to grow various crops on the rocks may result in a changing micro-climate and altered hydrology, both potentially affecting the toad’s survival, especially during the dry season when humid areas are rarer. In addition, the close proximity to a human settlement and to cash crop plantations may expose the toads to agrochemicals and other potentially harmful pollutants.

As the species could not yet be recorded from any other site, the only known population deserves particular attention. Schiøtz and Tandy (2004) already wrote that the species “is intrinsically at risk because of its restricted range”. We thus urge, and agree with Schiøtz and Tandy (2004), for setting up a continuous monitoring program of this population and suggest aiming for a protection status for the Idanre Hills in order to assure the long-term survival of Perret’s toad.
Acknowledgements

We thank the Chiefs and town elders of Idanre, as well as our field assistant Mr. Layi, allowing access to their community land. ABO also wishes to thank his wife, Mrs. F. Onadeko, who partly funded the survey costs. A. Schiøtz permitted the use of the photos in Fig. 1.

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Appendix

Specimens investigated

Amietophrynus perreti: ZMB 80331 (female, 61.1 mm SVL), Nigeria, Ondo State, Idanre Hills, 7°6’ 32.28”N, 5°6’45.3”E, 13 March 2014, GenBank#: KM219928, from a bush along a small stream flowing down the rock, leg. A.B. Onadeko; ZMB 80332 (female, 64.4 mm), 7°8’4.2” N, 5°6’2.1”E, all other data as in ZMB 80331; ZMB 80333 (cf. female, 55.3 mm), 14 March 2014, 7°6’44.62”N, 5°6’34.62”E, GenBank#: KM219927, all other data as in ZMB 80331; ZMB 80334 (female, 60.5 mm); ZMB 80335 (male 40.2 mm); ZMB 80336 (male 39.4 mm); ZMB 80337 (male, 36.6 mm); 26–28 October 2013, all other data as in ZMB 80331.

Amietophrynus regularis: ZMB 80338 (female, 65.3 mm) & ZMB 80339 (male, 64.6 mm), 26–28 October 2013, all other data as in ZMB 80331.
“TUBULAR GILLS” Extreme gill modification in the Thyasiroidea with the description of Ochetoctena tomasi gen. et sp. nov. (Bivalvia: Thyasiroidea)

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Abstract

Three dimensional tubular structures of the ctenidium of some thyasirid bivalves are described for the first time. The classification of the thyasirid gill is modified accordingly into five types based on the number of demibranchs, reflection of the filaments and shape of the filaments, either rod, laminar or tubular. The tubular structure is seen in its most modified form in a chemosymbiotic abyssal species from the south-east Atlantic, which is described here as Ochetoctena tomasi gen et sp. nov.

Key Words

Gill morphology
Thyasiroidea
New genus
Regab pockmark

Introduction

Chemosymbiotic bivalves typically host their autotrophic bacteria in tissues of the ctenidium (gill). As a consequence gill morphology is modified and from a gross perspective these gills are thick and fleshy in comparison with those adapted for filter feeding (Taylor and Glover 2010). The fleshy nature of the gills of thyasirids and lucinids was recognised long before their function was discovered and was shown to be a result of elongation of the abfrontal zone of the filaments (Allen 1958). Bacterial symbiosis associated with this modification was confirmed by a number of studies in the 1980s (see review by Taylor and Glover 2010). In the heterodont families Lucinoidea and Vesicomyidae the association is apparently obligate but the Thyasiridae display both symbiotic and asymbiotic taxa. Variation in thyasirid gill structure was first demonstrated by Southward (1986), and later in a wider ranging study by Dufour (2005). Dufour (2005) classified thyasirid gills into three categories based on the number of demibranchs, the degree of abfrontal extension and the extent of bacterial symbiosis. In all three types the filaments were either rod-like or lamellar, the lamellar form having greatest abfrontal extension and strongest association with chemosymbiotic bacteria. This lamellar gill (type 3 of Dufour 2005) has recently been described from two other heterodont families the Montacutidae (Oliver, Southward and Dando 2012) and the Basterotiidae (Oliver 2013).

A more complex gill, akin to the tubular form of many lucinids (Distel and Felbeck 1987) has been suggested to occur in Conchocele (Taylor and Glover 2010; Dufour 2005) and as such was partially described by Nakazima (1958) in C. disjuncta Gabb (= C. bisecta Conrad). In thyasirids the bacteria are extracellular with the exception of “Maorithyas” hadalis where an intra-cellular arrangement was described and where the frontal cilia are absent (Fujiwara et al. 2001).
Recently I was sent two thyasirids from the Regab pockmark (Gulf of Guinea) to identify and name following their inclusion in the bacterial study of Rodrigues and Duperron (2011). From a cursory examination it is immediately apparent the gill filaments are not laminar but that the gill is a three dimensional network of tubules.

Using scanning electron microscopy this paper describes the gross structure of these “tubular” gills and compares them with those described by Dufour (2005), especially the lamellar (Type 3) gill associated with chemosymbiotic taxa. Both Conchocele and the Regab thyasirid have been shown to be chemosymbiotic (Imhoff et al 2003; Rodrigues and Duperron 2011). Comparisons with the lucinoid gill are made and finally the Regab thyasirid is described as Ochetoctena tomasi gen. et sp. nov.

Materials and methods

Specimens examined

Thyasira sarsi (Philippi, 1845). North Sea:- Lyell Oil Field, 60°55’N, 01°14’E, 150 m, National Museum of Wales, 20+ specimens.

Thyasira flexuosa (Montagu, 1803). North Sea, National Museum of Wales, 20+ specimens.

Axinus cascadensis Oliver & Holmes, 2007. Baby Bare Seamount, Cascadia Basin, 2591m. National Museum of Wales, 5 specimens.

Conchocele bisecta (Conrad, 1849). Locality unknown, probably Queen Charlotte Sound, British Columbia. RBCM 006-00076-001, 1 specimen.

Undescribed new genus, single specimen, Off Quatsino Sound, Vancouver Island, British Columbia, Canada, 50°15.482’N, 128°26.400’W to 50°14.519’N, 128°26.567’W, 1086 – 1318m. Coll. J. Boutillier, Fisheries and Oceans Canada, 02 September 2004. RBCM 010-00221-005.

Conchocele excavata, (Dall, 1901). Farallon Islands, San Francisco County, California, USA, Station J-13 (Benthos 9). SBMNH 83964, 1 specimen.

Ochetoctena tomasi gen. et sp. nov. (this paper), Regab Pockmark, Gulf of Guinea, West Africa, RV Meteor GUINECO cruise, M76/3, Dive 215, 05°47.84’S. 09°42.64’E. 3167 m, 2 specimens.

Parathyasira resupina Iredale, 1930. Holotype, specimen not examined, image from Australian Museum, Sydney. Malacology:C.57834.

Parathyasira subcircularis (Payne & Allen, 1991). Rockall Trough, Scotland, North-east Atlantic, 58°26’N 12°42’W, 1595 m, NMW.Z. 1 specimen.

Parathyasira sp. nov. Owen Basin, Arabian Sea, 19°08’N 58°39’E, Discovery Cruise 211—stn12719#1, 3150m, NMW.Z.1995.009. 1 specimen.

Methods

All specimens had been previously fixed in ethanol or formaldehyde of unknown concentrations. For scanning electron microscopy, gill tissues were excised and cut transversely and longitudinally using a thin razor blade. Tissues were dehydrated in 100% ethanol overnight and critically point dried with liquid CO2 as the intermediate fluid in a Quorum K850 critical point dryer. Dried samples were mounted and gold coated before examination using a Jeol Neoscope SEM.

Institutional Abbreviations

AMS – Australian Museum, Sydney
NMW.Z – National Museum of Wales
RBCM – Royal British Columbia Museum
SBMNH – Santa Barbara Museum of Natural History

Results

The laminar filament. (Type 3L) in Thyasira flexuosa, T. sarsi, Axinus cascadensis, “Conchocele” excavata and the undescribed genus from Quatsino Sound

Both demibranchs are present, the outer extending over approximately half of the inner (Figs 1A-C). The filaments are fully reflected forming a descending and ascending lamella. The filaments are extended abfrontally giving each a laminar form. The supra-branchial chamber is represented by the large dorsal space (sbc) between the descending and ascending laminae. Ventrally the abfrontal regions extend and fuse to form continuous inter lamellar septae of varying patterns (ils) (Figs 1A-C, 2A, B). Using Thyasira sarsi as typical of this group the micro-structure is as follows. All parts of the abfrontal surfaces and the septae (ils) are lined with polygonal bacteriocytes (Fig. 2B). The frontal face of the filaments are fully ciliated (Figs 2C, D) with a median band of frontal cilia (fc) bordered on both sides by a band of lateral frontal cirri (lfc) and behind these a band of lateral cilia (lc). If removed the ciliated surfaces can be recognised by a remaining intricate pattern of scars on the epithelial surfaces (Fig. 2E, arrowed). Immediately behind the ciliated bands there is a network of inter-filament junctions (ifj) (Fig. 2D).

In “Conchocele” excavata only alternate filaments fuse to form inter lamellar septae and the middle portions of these septae do not bear bacteriocytes (Fig. 2F).

The tubular/laminar filament (Type 4T/L) in Conchocele bisecta

Both demibranchs are present, the outer and inner of approximate equal size (Fig. 1D). The filaments are fully reflected forming descending and ascending lamellae and appear to be narrowly separate along their entire length. At low magnification the filament is seen to be extended abfrontally but rather than appearing to laminate the frontal region appears as a series of small blocks but these are not apparent abfrontally. Under the SEM the frontal surface appears as a series of vertical bands with rows
of openings between the bands (Fig. 3A, openings white arrows) The vertical bands represent the frontal face of each filament and are strongly angulate with no trace of ciliation (Figs 3E, F). Nakazima (1958) assumed that the cilia had been lost through poor preservation but if this was the case the intricate pattern of ciliary ‘roots’ would remain and be visible under the SEM (see Fig. 2E for comparison). The lateral margins of the frontal bands are smooth (Fig. 3E) but between each band a small projection can be seen sitting on the inter filament junction (Fig. 3F, arrowed).

With the frontal bands ripped off a regular tubular structure is seen (Fig. 3B) with inter filament junctions (arrowed). Viewed from the abfrontal face of a lamella the regular tubular structure is not apparent and replaced by laminar filaments (Fig. 3C abf-lam). Widely separated but torn inter lamellar junctions (ilj) are visible in Fig. 3C, and best seen in cross-section in Fig. 3D (ilj). In cross-section (Figs 3D, 4) it can be seen that the frontal zone (fz) is narrow consisting primarily of the angular bands. The tubular zone (tbz) is a little over half the thickness of the lamella with the remainder being laminar (lz). Inter-lamellar junctions maintain a series of inter lamellar spaces (Fig. 3D, ils). Behind the frontal zone and between the laminar and tubular zones are inter filament junctions (iffj).

The tubes are lined with densely packed bacteriocytes as is the surface of the laminar zone, the inter lamellar junctions do not bear bacteriocytes. Preservation was not sufficient to acquire detailed images of the bacteria.

The tubular filament (Type 5T) in *Ochetoctena tomasi*

Both demibranchs are present but the outer is about half the depth of the inner (Fig. 1E). The normal filament structure is not apparent even under low magnification but appears as a series of small blocks (Fig. 8F). In transverse view (Figs 5, 7C) the ascending and descending arm of each filament is seen to be made up of a series of tubules (lt), abfrontally these are fused to form a median tube (mt). There is a small dorsal supra branchial chamber (Fig. 5 sbc). In lateral section the tubes can be seen to open between the frontal faces of the filaments (Fig. 6 te, 7B white arrows). These faces (Fig. 7B black arrows) are composed of a double band of the bases of lateral frontal cirri. Although not well preserved there are indications of lateral cilia and the frontal cilia zone is a deep groove. With the frontal zone ripped off the tubes can be seen in cross-section (Fig. 7D) and long section (Fig. 7E) if cut laterally. The walls are composed mostly of bacteriocytes (bct), the lumen is open and attached to the walls are numerous spherical cells (sph) of unknown function but may be involved with the elimination of waste products as suggested for similar structures observed by Reid and Brand (1986). Sporadically there are bundles of filaments (Fig. 7F ptf), 15 - 20 μm in length, each with a swollen, paddle-shap tip. These are similar in form to structures described as hyphomicrobial cells observed in *Syssomatina* by Oliver et al. (2013). This interpretation is open to question given the observation of paddle shaped artifacts observed on bivlace cilia by Beninger, Potter & St-Jean (1995). Here these structures are not attached to the walls of the tubules and if they are cilia they are probably not in situ.

The bacteria in the bacteriocytes are small, subspherical measuring approximately 0.65 μm in diameter (Fig. 7F bct).

Discussion

The results presented here reveal further modification of the thyasirid gill towards a complex three-dimensional structure. The gill types described by Dufour (2005) can now be added to with two additional types; “tubular/laminar”
Figure 2A–E. Scanning electron micrographs of the ctenidium of *Thyasira sarsi*; A gross transverse section, B longitudinal section of ventral portion of inner demibranch, C ciliated frontal surface, D transverse section through the frontal zone. E frontal surface with cilia removed. Figure 2F “*Conchocele* excavata”, longitudinal section of ventral portion of inner demibranch. abs abfrontal surface; fc frontal cilia; lc lateral cilia; lfc lateral frontal cirri; ifj inter filamental junction; ils inter lamellar septum; sbc supra branchial chamber.
Figure 3. Scanning electron micrographs of the ctenidium of *Conchocele bisecta*; A frontal surface, openings of tubules arrowed; B frontal surface removed to reveal tubules; C abfrontal surface of laminar filaments; D longitudinal section; E edge of a frontal face; F section of the frontal face with projection on the inter filamentar junction arrowed. abf lam abfrontal surface; fz frontal zone; ils inter lamellar space; ilj inter lamellar junction; lz laminar zone; tbz tubular zone.
and “tubular”. The known gill types can be summarized as follows (Table 1) with a revised nomenclature based on that of Dufour 2005. The revised nomenclature incorporates the shape of the filaments and the number of demibranchs. Filaments that have no or little abfrontal extension are termed rods and these equate to Dufour Types 1 and 2. For Dufour Type 2 those with single demibranchs and non-reflexed filaments are termed “Type 2b (R)” while those with both demibranchs are termed “Type 2a (R)”. The letters “L” and “T” refer to the laminar and tubular structures respectively. For each gill type the corresponding genera are listed, but some remain unallocated due to lack of data.

The extent of frontal ciliation is noted but the present observations give rise to difficulties in interpretation of the functioning of the tubular/laminar and tubular gills. All Type 3 (L) gills have complete ciliation of frontal and lateral cilia with eulateral cirri as also seen in the Lucinoidea (Taylor and Glover 2010). Here, it has not been possible to show any similar ciliation in Conchocele (Type 4T/L). Without ciliation it is difficult to comprehend how the currents to drive the water flow through the gill tubules are generated. Bernard (1972) records ciliary currents over the gill but gives no details of the ciliation. The lack of the basal structure of the cilia and cirri usually seen when the cilia are removed suggests that the Conchocele gill is atypical but its functioning remains enigmatic and requires observation on live material. It seems improbable that the small projections sitting on the frontal interfilamentar junctions, even if motile, could drive water currents into the tubules and could not produce the currents indicated by Bernard (1972).

Although there is an indication of frontal ciliation in Ochetoctena this is reduced and in both this genus and Conchocele they may be unable to create sorting and feeding currents on the gill.

Thyasirids typically hold their bacteria extra-cellularly (Dufour 2005) but the taxon Maurithyas hadalis is reported to hold the bacteria intra-cellularly (Fujiwara et al 2001). This unique condition suggests a further gill type

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**Figure 4.** Scanning electron micrograph of the ctenidium of *Conchocele bisecta* showing details of the frontal, tubular and laminar zones. fz frontal zone; ifj (abf) inter filamentar junctions, abfrontal; ifj (f) inter filamentar junctions, frontal; lz laminar zone; tbz tubular zone.

**Figure 5.** Scanning electron micrograph of the ctenidium of *Ochetoctena tomasi*, lateral view of a filament. abf (free) free abfrontal surface; att attachment point; fs frontal surface; lt lateral tubule; mt median tubule; sbc supra branchial chamber.
Figure 6. Scanning electron micrograph of the ctenidium of *Ochetoctena tomasi*, oblique section. fil frontal edge of a filament; lt (asc) lateral tubule of ascending arm; lt (dsc) lateral tubule of descending arm; mt median tubule; te tubule entrance.

but the gross structure of the gill remains undescribed. The allocation of this species to *Maorithyas* is incorrect as both shell and anatomy are not in agreement. The type species of *Maorithyas, M. marama* Fleming, 1950 is illustrated by Oliver and Sellanes (2005) and can be seen to be similar to *Thyasira sensu stricto* in both shell and anatomy.

This tubular structure increases the surface area of the bacteriocyte zone and creates a more rigid network further facilitating the movement of water from the infra-branchial chamber to the supra-branchial chamber. Similar structures are present within the Lucinoidea; Dando et al (1985) noted the inter-lamellar bridges in *Myrtea spinifera* and Distel and Felbeck (1987) illustrated a complex tubular structure in *Lucinoma aequizonata*. Such complex structures are found throughout the Lucinoidea but this gill differs in consisting of a only a single demibranch (Taylor and Glover 2010). The tubular structure is yet another convergent morphological feature shared by chemosymbiotic thyasirids and lucinids.

The gill structures described here can potentially impact on the systematics of the Thyasiroidea. The observations on the gills of *Conchocele bisecta* and “C” *excavata* indicate that these taxa are not congenereic and this is part of the subject of another paper that also describes a new genus containing species previously assigned to *Conchocele* (Oliver and Frey, in press).

**Table 1.** Classification of gill types found in the Thyasiroidea, modified from Dufour (2005).

| Revised type | Dufour type | Demibranchs | Reflection | Abfrontal extension | Frontal ciliation | Filament Form | Genera |
|--------------|-------------|-------------|------------|---------------------|------------------|---------------|--------|
| Type 1R      | Type 1      | 2           | +          | –                   | Complete         | Rod           | Axinopsida |
| Type 2aR     | Type 2      | 2           | +          | –/+                 | Complete         | Rod           | Parathyasira, Thyasira s.l. |
| Type 2bR     | Type 2      | 1           | –          | –                   | Complete         | Rod           | Axinulus, Adontorhina, Mendicula, Leptaxinus, Genaxinus |
| Type 3L      | Type 3      | 2           | +          | ++                  | Complete         | Laminar       | Thyasira ss, Maorithyas, Axinus, Spinaxinus, Channelaxinus |
| Type 4T/L    |             | 2           | +          | ++                  | Absent?          | Tubular/laminar | Conchocele |
| Type 5T      |             | 2           | +          | +++                 | Reduced          | Tubular       | Ochetoctena |
Figure 7A–F. Scanning electron micrographs of the ctenidium of *Ochetoctena tomasi*. A frontal face, frontal surface intact on the left, removed on the right to reveal tubules; B frontal surface with entrances to tubules arrowed; C single filament showing lateral and median tubules; D cross section of a single tubule; E long section of adjacent tubules; F bacterial bundles within bacteriocytes and paddle tipped filaments in the lumen of the tubule. bct bacteriocyte; sph spherical body; ptf paddle tipped filaments; fs frontal surface; fs (rem) frontal surface removed; lt lateral tubule; mt median tubule.
**Figure 8 A–F.** *Ochetoctena tomasi*, A–B external and internal views of the shell of the holotype, NMW.Z.2014.014.0001; C–E scanning electron micrographs of the shell surface; F gross anatomy viewed from the left side after removal of the mantle. aa anterior adductor muscle; f foot; lbp lateral body pouch; id inner demibranch; od (r) remanant of outer demibranch; pa posterior adductor muscle.

**Systematics Appendix**

**Class** Bivalvia Linnaeus, 1758  
**Subclass** Heterodonta Neumayr, 1884  
**Order** Veneroida H&A Adams, 1856  
**Superfamily** Thyasiroidea Dall, 1900  
**Family** Thyasiridae Dall, 1900

**Genus** *Ochetoctena* gen. nov.  

http://zoobank.org/1063B2E8-1D45-49CB-BB5F-2B12FD047BE9

**Type species:** *Ochetoctena tomasi* sp. nov. this paper.  
Monotypic.

**Definition.** Equivalve, Equilateral. Outline subcircular, lunule margin depressed, posterior margin with a single weak sinus. Posterior sulcus shallow but prominent. auricle absent. Escutcheon narrow, shallow. Ligament deeply sunken. Hinge edentulous. External sculpture of commarginal lines and growth stops, overall with microscopic conical, calcareous spines these randomly distributed with some linked by low ridges. Ctenidia of two demibranchs, filaments tubular, “Type 5T”.

**Etymology.** *Ochetoctena*, meaning tube-gilled from *ochetos* Greek “a water pipe or conduit” and *ktenos* Greek, “a comb” and the root for ctenidium (Brown 1956).

**Distribution.** Known only from the Regab pockmark off west Africa.
Ochetoctena tomasi sp. nov.

http://zoobank.org/BEADD75-67E5-4141-901B-E28EEBD1941
NMW.Z.2014.014.00001

Thyasira n. sp. Regab – Rodrigues & Duperron, 2011.

Material examined. 2 specimens (1 broken) in ethanol. Regab Pockmark, Gulf of Guinea, West Africa, RV Meteor GUINECO cruise, M76/3, Dive 215, 05°47.84’ S. 09°42.64’ E. 3167m

Holotype, entire shell from above, NMW.Z.2014.014.00001

Description of holotype. 14.4 mm long, 13.4 mm high, 4.3 mm half t. Equivalve, Equilateral. Outline subcircular. Anterior dorsal (lunule) margin concave, lunule slightly depressed, ovate, smooth, demarcated by a low ridge. Posterior dorsal (escutcheon) margin almost straight; escutcheon narrow, very shallow,

Figure 9. Shells and anatomy of granulose thysirids of the genus Parathyasira. A–E an undescribed abyssal species from the Arabian Sea, NMW.Z. 2014, A–B external and internal views of the shell; C–D scanning electron micrographs of the granulose microsculpture; E gross anatomy. F Parathyasira resupina, holotype and type of the genus, New South Wales, AMS C57334; G–I Parathyasira subcircularis, Rockall Trough, NMW.Z.2013, G external of shell; H gross anatomy; I micrograph showing radial granulose sculpture.
auricle lacking. Posterior ventral margin weakly indented corresponding with a distinct but shallow posterior sulcus. Median area slightly flattened corresponding with slightly straightened ventral margin. Anterior ventral and anterior a continuous broad curve. Ligament prominent but deeply sunken, about half the length of the escutcheon. Hinge edentulous. Sculpture of irregular commarginal lines and growth stops, overall with microscopic conical, calcareous spines emerging through the periostracum, these as isolated individuals, in small clumps or connected by weak sub-concentric ridges. Periostracum relatively thick, brown in colour, shell white. Muscle scars prominent, anterior adductor scar elongate not diverging from the broad pallial line; posterior adductor scar oval.

Anatomy. Mantle edge thick, free except for a junction demarcating the posterior aperture. Foot large, vermiform, toe elongate, heel very small. Lateral pouches large, multi-lobed, lobes cuboid. Ctenidium of two demibranchs, the outer about half the length of the inner; both with fully reflected filaments, these of Type 5T, composed of tubules fused to a median tube with only a small dorsal inter lamellar space.

Etymology. Named for my son Tomas.

Discussion. The shell of Ochetoctena has a weak posterior sulcus and the escutcheon is excavated but lacks any auricle to support the sunken ligament. In this it differs from Thyasira sensu stricto where there is a well developed auricle and from Conchocele which is oblique with a very strong posterior sulcus. Species lacking an auricle are often placed in the genus Parathyasira (Oliver and Killeen 2002; Payne and Allen 1991). The type species of Parathyasira is P. resupina Iredale, 1930 (Fig. 9F) and has a shell microsculpture of radial rows of calcareous spines. This character is also seen in the Atlantic species P. granulosa (Monterosato, 1874), P. subcircularis Payne & Allen, 1991 (Fig. 9G-I) and an un-named species from the Arabian Sea (Figs 9A-E). The spines in these species are angular with strongly developed basal connections between the rows (Figs 9C-D). In contrast the spines in Ochetoctena are not arranged in rows, are conical and the basal connections are weak or absent (Figs 8C-E). The genus Spinaxinus is characterised by having a spiny microsculpture but this is entirely periostracular (Oliver and Holmes 2006; Oliver et al 2013).

Ochetoctena is the only thyasirid known to have etenidia of the Type 5T structure; Conchocele has a partly tubular gills of the Type 4TL morphology. The etenidia of these Parathyasira species show poorly developed abfrontal extension resulting in a flimsy open structure. Dufour (2005) reported that the gills of P. granulosa are of her type 2 (type 2aR above) suggesting that the genus Parathyasira is characterised by a weak symbiotic partnership.

There are, therefore, shell and anatomical synapomorphies that separate Ochetoctena from all other known thyasirids.

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Abstract
A new specialist driftwood talitrid from the Swale, U.K., is figured and described as Neotenorchestia kenwildishi gen. n., sp. n. A further new driftwood talitrid, Macarorchestia pavesiae sp. n., is figured and described from coastal regions in the Adriatic Sea. Orchestia microphthalmalma Amanieu & Salvat, 1963 from the Atlantic coast of France is re-designated as Macarorchestia microphthalmalma (Amanieu & Salvat, 1963). A key is provided for the known species of driftwood talitrids in northeastern Atlantic and Mediterranean coastal regions.

Key Words
Neotenorchestia kenwildishi gen. n., sp. n.
Macarorchestia pavesiae sp. n.
Macarorchestia microphthalmalma (Amanieu & Salvat, 1963) comb. n.

Introduction
Driftwood specialist hoppers are a rare, difficult to find, ecological group of Talitridae which are virtually confined to rotting driftwood where they live in galleries, consuming rotting driftwood and reproducing with relatively small broods (1 to 19 ova per brood in the Macarorchestia so far described). The ova are incubated in the brood pouch formed by the oostegites on peraeon segments 2 through 5 and hatch as juvenile forms. Because talitrids have no larval stage their dispersal, particularly to distant oceanic islands, was seen as problematic. The general view was that some form of passive rafting dispersal was involved (Wildish 1988). In the lineal “island” theory (Wildish 2012) this view was focussed and the hypothesis proposed that driftwood hoppers, with near-permanent residence in driftwood, were important agents of long distance dispersal for talitrids, particularly to distant oceanic islands. Circumstantial evidence supporting the lineal “island” theory was presented in Wildish (2012), showing that driftwood talitrids reached the northeast Atlantic islands on driftwood transports. After reaching the remote, recently-formed, volcanic islands they evolved further there into terrestrial or cavernicolous forms if sub-tropical rain forest or seashore caves were contiguous habitats with the supralittoral and also lacked a talitrid fauna. Alternatively driftwood specialists remained in place in their primary ecotope as supralittoral, driftwood hoppers if the supply of driftwood habitat was plentifully available.

Currently known driftwood taxa (Wildish et al. 2012) comprise 5 species presently grouped in 2 genera from the Mediterranean and northeast Atlantic (Macarorchestia and Orchestia), with another genus (Platorchestia) reported from the west coast of North America by Bousfield (1982). The preponderance of driftwood hoppers in the northeast Atlantic/Mediterranean coastal areas is suggested to be the result of more intensive sampling there.
Other geographic areas and particularly the southern hemisphere, where no driftwood hoppers have yet been found, have not been sampled intensively enough to conclude that they are without driftwood talitrid specialists.

As a result of molecular studies of *Macarorchestia* *remyi* it was suggested that two genetically distinguishible forms: one centred in the Tyrrhenian and the other in the Adriatic Sea were present (Pavesi et al. 2011). This result was confirmed with further relative growth studies (Wildish et al. 2012) and a new species referable to *Macarorchestia* is described herein. Molecular and relative growth studies (Wildish et al. 2012; Pavesi et al. 2014) determined the taxonomic identity of an unknown talitrid taxon found in a driftwood log which stranded in the Swale, U.K., and found morphological evidence supporting a generic level change in nomenclature for *Orchestia microphtalma* Amanieu & Salvat, 1963. A formal presentation of the taxonomic changes outlined above is provided with a key for the currently known driftwood species from the Mediterranean and northeast Atlantic coastal region.

**Material and methods**

Slides were prepared as temporary mounts without staining and after dissecting mouthparts and limbs. Some were prepared as permanent mounts by Sara LeCroy, after staining with lignin pink and permanently mounted in CMCP-10 (Master’s Company, Inc.).

Photographs of limb parts were made with a Carl Zeiss photomicroscope and digital Canon 990 camera. Adobe Photoshop (version 7.0), Illustrator (version 11.0) and a Wacom tablet were used to draw limb parts and prepare plates, essentially as outlined in Coleman (2003, 2006). Antenna, mouth and limb part abbreviations used throughout are: A1 = first antenna, A2 = second antenna, LL = lower lip (labium), UL = upper lip (labrum), RMnd = right mandible, LMnd = left mandible, Mx1 = first maxilla, Mx2 = second maxilla, Mxp1 = maxilliped, G1 = first gnathopod, G2 = second gnathopod, P3 to P7 = pereopods 3 through 7, P11 to P13 = pleopods 1 through 3, Up1 to Up3 = uropods 1 through 3, and T = telson. Body length was measured accurately from digital photographs of each individual pinned in a dissecting dish. The total body length (TBL) was measured from the most anterior part of the cephalon to the telson tip, on digital photographs. Body length was determined by measuring up to 5 articles of the first antenna with up to 5 articles (versus 3 in Stock). The pleopods are biramous with a basis which is not reduced, but both rami are variously reduced dependant on the total body length of the largest adults of the species. Thus in the smallest species, *martini*, there is no segmentation in the rami and 1–3 plumose setae and in the largest, *microphtalma*, there up to 5 segments and 11 plumose setae. The pleopod rami may be sexually dimorphic, as in male *remyi*, and the second antennal flagellum articles are sexually dimorphic in female *M. pavesiae* sp. n. In addition all species are small, that is to say < 15 mm in total body length and lack epidermal pigment patterns.

**Macarorchestia martini** Stock, 1989

Stock 1989: 1109.

**Material examined.** Male holotype and 8 females (allosome and paratypes) on loan from Zoological Museum of Amsterdam, Amsterdam, the Netherlands (AMPH. 108.57). Collected by J. Stock on 2 August, 1987 from Gruta das Aguihas in Porto Judeus on the island of Terceira, Azores archipelago.

**Distribution.** Known only from the type locality on the island of Terceira.

**Epidermal pigment patterns.** Not mentioned by Stock (1989).

**Remarks.** This is the type species and smallest *Macarorchestia*. Stock (1989) was equivocal about whether this was a recent troglobiont specialist or trogloxenous form. I concur with the latter view and believe that the adaptations (small body length, body length greater in the female, small eyes, reduced pleopod rami and shortened length of the first 5 pereopods) indicate that *M. martini* is a driftwood specialist.

**Macarorchestia roffensis** (Wildish, 1969)

Wildish 1969: 288; Lincoln 1979: 212; Wildish 1987: 571; as *Orchestia roffensis* or *O. roffensis*; Ruffo 1993: 739.

**Material examined.** Male holotype (BMNH 1968:64), one female allotype (BMNH 1968:65) and 416 paratypes of all life history stages (BMNH 1968:66). Collected by D.J. Wildish(DJW) in August 1968, 0.2 km upstream from
Chatham Ness in Limehouse Reach, Medway estuary, near Rochester, Kent, U.K.

**Distribution.** Besides the type locality one other locality in the Medway estuary was found in 1968 with a few specimens in a driftwood log by DJW. The location was ~0.6 km upstream from the old Rochester Bridge in Tower Reach on the eastern shore. A few specimens collected by DJW in 1999 from the Swale, in Ferry Reach ~0.5 km northwest of Kingsferry Bridge on the mainland shore.

**Epidermal pigment patterns.** Absent.

**Remarks.** This is the second smallest *Macarorchestia*. The type locality was destroyed during reclamation of Frindsbury marsh as an industrial estate.

*Macarorchestia remyi* (Schellenberg, 1950)

Schellenberg 1950: 325; Ruffo 1993: 739.

**Material examined.** Two males, 4 females, 1 juvenile (Crust. 25468) ZMB Museum für Naturkunde, Berlin. Collected by A. Schellenberg from a seashore cave at St. Barthelemy, Corisca. A total of 47 individuals (combined sample) of all life history stages, including females with ova, collected by L. Pavesi on 20 January and 15 April 2007 at Principina a Mare, Tyrrhenian Sea, Italy. Sample location co-ordinates: 42°41'18"N, 10°59'53"E. A further sample collected at Principina a Mare by L. Pavesi on 24 April 2011 consisted of 28 individuals of all life history stages, including females with ova. One individual from Corfù Island (Ionian Sea, Greece) collected by L. Pavesi in 2007 (Destructively used in DNA analysis).

**Distribution.** Besides the locations found by L. Pavesi for this species, published records include that of Ruffo (1960) in Sardinia and Ruffo (1993) in France, Greece and Italy. Possibly some of these records refer to the species described below.

**Epidermal pigment patterns.** Absent.

**Remarks.** This is the second largest *Macarorchestia*. In recent times the type locality has been destroyed by cleaning beaches for the benefit of tourists (L. Pavesi, pers. comm.).

*Macarorchestia pavesiae* sp. n.

http://zoobank.org/42B57D1C-E898-408E-AA2F-0F7A27915691

Pavesi et al. 2011: 220; Wildish et al. 2012: 2678, as *M. remyi*.

**Type material.** Holotype male of 8.0 mm TBL (NHMUK 2014. 408) and allotype female of 7.5 mm (NHMUK 2014. 409) collected by L. Pavesi on 31 October, 2006 at Lesina, Adriatic Sea, Italy. 13 paratypes collected on the same date (NHMUK 2014. 410 - 419). Paratypes have also been deposited in the Natural History Museum, Verona and Museum at Rome University, Rome, Italy by L. Pavesi.

**Type locality.** Lesina, Adriatic Sea, Italy (41°54’11"N, 15°26’50"E).

**Other material examined.** 17 individuals collected by L. Pavesi in April 2006 at Varano, Adriatic Sea, Italy (41°55’12"N, 15°47’29"E).

**Diagnosis.** *M. pavesiae* sp. n. is distinguished from its close relative, *M. remyi*, by its smaller size, sexually dimorphic second antennal flagellum articles in adult females and absence of sexual dimorphism in pleopod rami.

**Description.** Based on male paratypes: total body lengths in the range of 8.0 to 6.7 mm. Figs 1 and 2. The male holotype has similar morphology to male paratypes dissected in preparing the figures.

Head deeper than long (1: 0.7); eyes small, round, less than half the head length. Antenna 1 flagellum 5-articulate with tip just exceeding the junction of peduncle segments 4 and 5 of antenna 2. Antenna 2 short, flagellum 12-articulate, peduncle not incrassate.

Mouthparts. Lower lip with lateral lobes, minute setae on the inner clefts. Left mandible with 4-dentate lacinia mobilis and large molar process. Right mandible with the tip of the dentate incisor bilobed, 6-dentate lacinia mobilis. Maxilla 1 inner plate narrow with 2 terminal plumose setae, inner margin with long, fine setae; outer plate with a palp, apical robust setae curved and serrated on the inner edge. Maxilla 2 both plates equal in size, inner with a single, plumose seta, with shorter robust setae on the distal edge; outer plate with simple robust setae. Maxilliped inner plates with 3 stout teeth apically, inner, outer and palp distal edges covered with robust setae; palp large and 3-articulated.

Peraeon. Gnathopod 1 weakly subchelate with palmate lobes on propodus and carpus; dactylus as long as propodus lobe; the largest robust setae are present on the posterior edge of the merus. Gnathopod 2 subchelate with propodus and dactylus massively enlarged, dactylus drawn out in a short, blunt tip, its inner surface lacking fine setae. Ventral edge of each coxal plate rounded and with fine robust setae. Peraeopods 3 to 5 short, pereaeopods 6 and 7 longer, the latter just longer than uropod 1. Peraeopods 3 and 4 lack a dactylus notch ("pinched unguis"). Peraeopods 6 and 7 not sexually dimorphic in merus and carpus. Six distinctive tufts of long, slender setae originating from the propodus of pereopod 7 as follows: anterodistal (2 setae), distal (6 setae), anterior side of pereopod near the first insertion of robust, bifid-tipped setae (4 setae), then on the posterior side of the pereopod: first insertion of robust, bifid-tipped setae (2 setae), second (4 setae) and third (3 setae). The maximum length of the longest seta from the distal tuft was 144 µm (Fig. 3).

Pleosome. Pleopod basis not reduced and with a pair of hooked coupling spines, robust setae and fine marginal setae absent. All rami and rami are shorter than the basis. The second pleopod rami have 3 articles each bearing a pair of long, plumose setae: exopod -7, endopod -5.
Figure 1. M. pavesiae sp. n. Habitus of female allotype total body length (TBL) = 7.5 mm. Mouthparts, telson and uropods from male paratype. Mouthparts, Up3 and telson, scale = 0.1 mm, except Lmnd, Up1 and Up2 = 0.25 mm.
Figure 2. *M. pavesiae* sp. n. habitus of male holotype, TBL = 8.0 mm. Limb parts from male paratype. Scale = 0.25 mm.
Urosome. In uropods 1 and 2 the inner and outer rami are of similar length, with 2-4 apical robust setae and 1 or 2 inter-ramal robust setae, basis with 1 or 2 dorsal-ventral, robust setae distally. Uropod 3 basis longer than the ramus and with 2 large, robust setae dorso-laterally. Smaller robust setae at the ramus tip but lacks inter-ramal robust setae. Telson with a mid-dorsal groove and 6-7 dorso-lateral robust setae on each lobe.

Sexually dimorphic differences. Based on non-breeding adult female paratype of 7.3 mm total body length.

Gnathopod 1 without palmate lobes on propodus and carpus. Gnathopod 2 basis slender and with weak robust setae. Palmate lobes present on merus, carpus and propodus, dactylus small (described as “mitten-shaped”gnathopod 2). Pairs of non-ovigerous oostegites on pereopods 2 to 5. Adult females greater than 7.5 mm body length with no more than 11 antennal flagellum articles (versus up to 13 in males). The 6 tufts of long, simple setae on the propodus of pereopod 7 in males compares with 2 tufts, of sparser and shorter length setae in females.

Epidermal pigment patterns. Absent.

Etymology. The name honours Dr. Laura Pavesi who originally discovered and collected the new species during graduate studies at the University of Rome, Italy.

Diagnosis. There are three known locations for this species on the shores of the Adriatic Sea and one on Corfû Island, Ionian Sea.

Macarorchestia microptalma (Amanieu & Salvat, 1963), comb. n.
Amanieu and Salvat 1963: 390; Pavesi et al. 2014, as Orchestia microptalma

Material examined. Male holotype (No. 5-1963) and paratypes in the Museum National d’Histoire Naturelle, Paris. Collected in 1962 by Mr. C. Caussanel from Cap Ferret Point near Arcachon on the Atlantic coast of France. Paratypes also in L’Institut de Biologie Marine at Arcachon. Collection by DJW in the type locality on 11th September 1967 and deposited in the Natural History Museum, London (BMNH 1967 10.6.1-75).

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Diagnosis. M. microptalma is distinguished from M. remyi and M. pavesiae sp. n., by:

- Sexually dimorphic tufts of long, slender, simple setae from the propodus of pereapod P7, with no tuft at anterodistal position and 4 tufts on the posterior side of the propodus in males, versus a tuft at anterodistal position and 3 tufts on the posterior side of the propodus in males of M. remyi and M. pavesiae sp. n. (Fig. 3),
its larger size, and
- lack of sexual dimorphism in pleopod and second antennal characters. Pleopod sexual dimorphism was discovered by Wildish et al. (2012) in *M. remyi* where males grew at the same rate as juveniles throughout life, whereas females grew at a slower rate. A2 flagellum article sexual dimorphism was present in *M. pavesiae* sp. n., where adult females grew at a slower rate than juveniles and males (Wildish et al. 2012).

### Distribution
Known from the type locality and 3 other locations further south on the French Atlantic coast (Lagardere 1966).

### Epidermal pigment patterns
Absent.

### Remarks
The largest species of *Macarorchestia*.

#### Orchestia Leach, 1814
Leach 1814: 402; Bousfield 1982: 22; Lowry and Fanini 2013: 205.

**Type species.** *Orchestia gammarellus* (Pallas, 1766).

**Component species.** Since the erection of *Orchestia* Leach, 1814 the genus has been uncritically used to include many new species from around the World. In more recent times genera have been split off from *Orchestia* including: *Platorchestia* by Bousfield (1982), *Palmorchestia* by Stock and Martin (1988) and *Macarorchestia* by Stock (1989). Bousfield (1982) re-defined the range of *Orchestia*, limiting species to those found in the Atlantic/ Mediterranean region. I have further limited the geographic range of this taxon to the northeast Atlantic, the Mediterranean and Black Seas, but excluding the western Atlantic coastline of North America. The northeast Atlantic islands including: Canary, Madeira and Azore archipelagos are also included in the region. The northerly limit is arbitrarily set at the Arctic Circle (thus including Iceland) and the southern one at the Tropic of Cancer. Circumstantial evidence (Henzler and Ingolfsson 2008) supports the presence of *Orchestia gammarellus* on northwest Atlantic coastlines (as far south as Maine), as a result of recent, post glacial, synanthropic, dispersal from the northeastern shores of the Atlantic.

Taking only *Orchestia* species which occur within this newly defined geographic range and excluding those outside it, synonyms, and where the taxonomic or ecological status is unclear (inclusive of *O. kosswigi* Ruffo, 1949—which is figured and described in Ruffo (1993) but its ecological status remains unclear; *O. guerni* Chevreux, 1889 and *O. gambierensis* Chevreux, 1908), leaving a total of 13 species (Table 1). The placement of these 13 species in five clearly separate habitats is consistent with a polyphyletic origin for them and that we can expect further generic splitting of *Orchestia*. In fact Lowry and Fanini (2013) have recently proposed a revision of the genus *Orchestia* in which all the species belonging to freshwater and terrestrial rain forest leaf litter of the northeast Atlantic islands (columns 3 and 4 in Table 1) were removed to a newly created genus *Cryptorchestia*. *O. kosswigi* is also transferred to the new genus and these authors describe a new species referable to *Orchestia*. *O. xylino* Lowry & Fanini from the Mediterranean Sea. Recent molecular evidence (Pavesi et al. 2014) does not support the close genetic relationship required by Lowry and Fanini’s proposal between *cavimana* and the Atlantic islands endemic “*Orchestia*” listed in column 4 of Table 1. One of these taxa, *O. guancha*, was shown to be close genetically to *O. gammarellus*, confirming earlier work by Villacorta et al. (2008). Pavesi et al. (2014) also show that *O. montagui* and *O. stephenseni* are not closely linked genetically to the other species of *Orchestia* inclusive of the type species *O. gammarellus*, plus *O. mediterranea*, *O. aestuarensis* and *O. guancha*. With the transfer of *O. microptalma* to *Macarorchestia* herein, this reduces the habitats occupied by “*Orchestia*” to 4. The genetic findings of Pavesi et al. (2014) suggest the polyphyletic status of *Orchestia* and a generic level re-alignment like that shown in Table 2. Further genetic and taxonomic work is needed to include all the species listed in Tables 1 and 2.

### Diagnosis
An interim diagnosis is provided based on the type species, *O. gammarellus* from the Medway estuary, U.K., as listed in Table 2. This is because of the demonstration of polyphyly (Pavesi et al. 2014) within the older view of the genus *Orchestia* and because of the resultant taxonomic uncertainty regarding which of the taxa in Table 1 should be included within *Orchestia*. A diagnosis of the 5 genera listed by letter in Table 2 is delayed because the current COI phylogeny (Pavesi et al. 2014) does not include 6 species of “*Orchestia*” (indicated by brackets in

### Table 1
Ecological habitats occupied by species of *Orchestia* which are listed in WoRMS Editorial Board (2013) available from: World Register of Marine Species, http://www.marinespecies.org, accessed in 2013-06-26, and occurring in the northeast Atlantic (including offshore islands), Mediterranean and Black Sea region.

| Marine/estuarine supralittoral wrack | Marine/estuarine eulittoral wrack | Freshwater supralittoral wrack | Terrestrial rain forest leaf litter | Marine driftwood |
|--------------------------------------|-----------------------------------|--------------------------------|-----------------------------------|-----------------|
| *gammarellus*                        | *mediterranea*                    | *chevreuxi*                    | *guancha*                         | *microptalma*   |
| *stephenseni*                        | *aestuarensis*                    | *monticola*                    | *stocki*                          | *canariensis*   |
| *montagui*                           |                                   |                                |                                   | *gomeri*        |
Table 2. Ecological habitats and proposed generic groupings of species listed in Table 1, based on the molecular phylogeny (COI) of Pavesi et al. (2014). ? before a species name indicates that the ecological habitat given has not been confirmed. Brackets indicate absence of COI data for that species.

| Genus       | Marine/estuarine supralittoral wrack | Marine/estuarine eulittoral wrack | Freshwater supralittoral wrack | Terrestrial rain forest leaf litter |
|-------------|--------------------------------------|-----------------------------------|--------------------------------|-----------------------------------|
| Orchestia   | gammarellus                          |                                   |                                |                                   |
| Genus A     |                                       | mediterranea                      | ?aestuarensis                  |                                   |
| Genus B     | stephensi montagui ?(xylino)          |                                   |                                |                                   |
| Cryptorchestia |                                   | cavimana                          |                                |                                   |
| Genus C     |                                       | tuancha (?gomeri)                 | (?canariensis)                  | (?stock)                          |
| Genus D     |                                       |                                   |                                |                                   |
| Genus E     |                                       |                                   |                                | (?chevreux)                       |

Adult total body length up to 22 mm; dorsal pigment patterns present; eyes medium in size, approximately one quarter of head length; antenna 1 flagellum just reaching antenna 2 peduncle of article 4; antenna 2 sexually dimorphic, peduncle slightly incrassate in adult males and without ventral plate on peduncle article 3; upper lip without robust setae; mandible left lacinia mobilis 4 dentate; maxilliped palp 3 articulate, article 2 with well developed medial lobe; gnathopod 1 of male subchelate with palm equal to dactyl, carpus and propodus free and with rounded lobes covered with palmate setae; gnathopod 1 of female parachelate, without lobes on carpus and propodus; gnathopod 2 of male strongly subchelate, merus and carpus free, dactylus with blunted tip and is half the length of the enlarged propodus; gnathopod 2 of female, ovigerous oostegite long and wide with many, long, simple, marginal setae, basis expanded anteriorly; pereaeopods 3-7 cuspipidactylate; pereaeopods 5-7 lack slender setae lining the anterior margin of the dactyl; pereaeopod 7 sexually dimorphic, adult males with merus and carpus enlarged; distinctive tufts of long simple setae of long simple setae on propodus of pereaeopod 7 absent in both sexes; pleon segments 1-3 lacking vertical slits; pleopod rami slightly, or not, reduced; uropods without apical, spade-like robust setae, uropod 1 not sexually dimorphic, peduncle lacking well developed dorsolateral robust setae distally, outer ramus with marginal robust setae, uropod 2 rami equal in length, uropod 3 ramus shorter than peduncle; telson apically notched with 6-8 robust setae per lobe and shorter than uropod 3.

Component species. Monotypic.

Diagnosis. As in Orchestia and specifically similar to Orchestia mediterranea A. Costa 1853 except for:
- lack of dorsal pigment patterns (versus dorsal pigment patterns in O. mediterranea as in Wildish, 1987).
- smaller size by neoteny (largest total body length estimated to be 12 to 16 mm, versus up to 20 mm in O. mediterranea)

Very similar to Orchestia mediterranea A. Costa, 1853 and if characterization is limited to conventional morphological methods this species can easily be misidentified as a juvenile O. mediterranea. The use of relative growth methods and regression fitting predictions as found in Pavesi et al. (2014, see Table 3) are required for definitive identification.

Etymology. Refers to the origin of the new genus by a form of neoteny and combination of the stem of this word with the genus Orchestia to which it is closely related.

Neotenorchestia kenwildishi sp. n.

http://zoobank.org/DD3AA445-5FAB-49FF-8D3E-84442454732C

Wildish et al. 2012: 2677; Pavesi et al. 2014, as unknown taxon.

Type material. Holotype-immature male of 7.2 mm total body length (NHMUK 2014. 397) and slide preparation from this individual (NHMUK 2014, 397). Nine juvenile paratypes (NHMUK 2014, 398 - 406) and 2 immature females (destructively sampled for temporary slide mounts and mtDNA analysis) removed from a cast-up driftwood log resting at the base of the seawall in the Enteromorpha zone by K.J. Wildish. The driftwood log had been tethered to an old cattle fence on the shore, so it could not float away. It was sampled on 14th June 2011, 23rd July 2011 and 13th August, 2011 from the Swale, near Kingsferry Bridge, U.K.

Neotenorchestia gen. n.

http://zoobank.org/D3C3762F-5DF0-47AD-9EC3-2DB079544F1E

Wildish et al. 2012; Pavesi et al. 2014, as unknown taxon.

Type species. Neotenorchestia kenwildishi gen. n., sp. n.
Figure 4. *Neotenorchestia kenwildishi* gen. n., sp. n. Habitus of juvenile paratype, total body length (TBL) = 3.7 mm. All other body parts from immature male holotype, TBL = 7.2 mm. Antennae and pleopod scale = 0.25 mm. Mouthparts, scale = 0.1 mm.
Figure 5. *Neotenorchestia kenwildishi* gen. n., sp. n. Immature male holotype as in Fig. 3, except peraeopod 3 is from a female paratype of TBL = 8.3 mm. Scale = 0.25 mm.
Type locality. Approximately 0.5 km west of Kingsferry Bridge, The Swale on the mainland shore in a single floating driftwood log (~ 3 × 0.5 × 0.5 m) determined to be of Douglas fir by Dr. P. Gasson, Kew (Pavesi et al. 2014). GPS position: 51°23.97’N, 00°43.56’E.

Maximum body length. Unknown, the largest found was an 8.9 mm TBL immature female. Mature adults predicted by relative growth methods to be 12 to 16 mm total body length (Pavesi et al. 2014).

Diagnosis. N. kenwildishi sp. n., can readily be distinguished from other driftwood hoppers of the genus Macarorchestia by its medium size eyes (versus small) and unreduced pleopods (rami sub-equal to basis, versus rami shorter than basis).

Description. Based on immature male holotype of 7.2 mm total body length. Figs 4 and 5.

Head deeper than long (1 : 0.5); eyes medium/large, round and greater than half the head length. Antenna 1 flagellum 4-articulate. Antenna 2 flagellum 13-articulate. Peduncle not incrassate.

Mouthparts. Upper lip with minute setae on the apical margin. Lower lip deeply cleft and with minute setae on the inner face. Maxilla 1 inner plate slim and with two terminal, plumose setae; inner margin with long fine setae; outer plate with a vestigial palp, api cal robust setae curved inwards, some simple and others serrated on the inner edge. Maxilla 2 with inner plate subequal to the outer, inner with a single, plumose seta and fine marginal setae below it; both inner and outer plates with long, simple robust setae which curve inwards. Left mandible with a 4-dentate lacina mobilis, 6-dentate incisor, strong molar process and setose accessory blades. Maxilliped with 3 strong apical teeth on the inner plate; inner, outer and palp edged with simple, robust setae; palp large and 3-articulated.

Peraeon. Gnathopod 1 weakly subchelate, palmate lobes on propodus and carpus. Gnathopod 2 weakly subchelate with dactylus shorter than the propodus lobe; palmate lobes on propodus, carpus and merus. Peraeopod 3 lacks a dactylus notch ("pinched unguis"), but this is present in an immature female. Peraeopod 5 shorter than pereaeopods 6 and 7, the latter not sexually dimorphic in the immature male.

Urosome. Uropod 1 rami subequal to peduncle. Peduncle with 2 rows of 2 robust setae. Terminal setae on each rami consists of 1 large and 1 or 2 smaller robust setae. 3 interramal robust setae on inner and 2 on outer ramus.

Sexually dimorphic differences. Based on immature female of 8.9 mm total body length.

In the absence of sexually mature males and females the only unique female characters found were: absence of palmate lobes on propodus and carpus of gnathopod 1, presence of pinched unguis on pereaeopod 3, and presence of small, rudimentary oostegites on coxae of pereaeopods 2-5.

Etymology. The name honours Kenneth J. Wildish who discovered and collected the new taxon in the Swale during the summer of 2011.

Epidermal pigment patterns. Absent.

Distribution. Known only from the type locality.

Key for northeast Atlantic/Mediterranean Macarorchestia and Neotenorchestia gen. n.

1 Medium size eyes and pleopod rami > basis length ................................................................. N. kenwildishi sp. n.
   - Small eyes and pleopod rami < basis length ................................................................. M. microphtalma
2 Maximum adult size (TBL < 8 mm), female TBL > male ........................................................ 3
   - Maximum adult size (TBL > 8 mm), male TBL > female .................................................. 4
3 Third pleopod exopod length ratio > 0.16 ............................................................. M. martini
   - Third pleopod exopod length ratio < 0.16 ................................................................. M. roffensis
4 Maximum adult size (TBL = 15 mm), male TBL > female ........................................................ M. pavesiae
   - Lacks sexual dimorphism in A2 and pleopods
   Males with no tuft at anterodistal and 4 tufts of long, simple setae on posterior edge of of P7 propodus
   - Maximum adult size (TBL < 13 mm), male TBL ≤ female .................................................. 5
   With sexual dimorphism either in pleopods or A2 flagellum articles
   Males with a single tuft at anterodistal and 3 tufts of long, simple setae on posterior edge of P7 propodus
5 Adult male TBL > 8 mm fit: $y = 0.050x + 0.159$ ................................................................. M. remyi
   - Adult male TBL > 8 mm fit: $y = 0.026x + 0.024$ ................................................................. M. pavesiae
   - Adult female TBL > 7.5 mm fit: $y_1 = 0.585x + 6.995$, ......................................................... M. remyi
   - Adult female TBL > 7.5 mm fit: $y_1 = 0.130x + 9.248$, ......................................................... M. pavesiae
   (where $x$ = TBL, $y$ = Pl3 Ex L, and $y_1$ = A2 FA)
Discussion

The following arguments were considered in deciding how to name the unknown taxon. Relative growth data was available to show that the unknown taxon fundamentally differed from juvenile *Orchestia mediterranea* (Wildish et al. 2012; Pavesi et al. 2014). The relative growth of the unknown taxon differed from that in *O. mediterranea* by:

- being slower growing with a reduced terminal moult size,
- sexualization beginning at an earlier moult stage and with fewer moult stages per life history, and
- dorsal pigment patterns being absent.

The first two of these three phenotypic characters are described as neotenous dwarfism and are the basic adaptations possessed by all driftwood specialist talitrids of the genus *Macarorchestia* Stock. Because the unknown taxon clearly does not belong to *Macarorchestia*: by possession of fully developed eyes and pleopods, by COI divergence differences (Wildish et al. 2012) and because it is clearly a driftwood specialist, both in habitat and adaptive morphology, the unknown taxon should be placed in a new genus.

On the other hand molecular evidence suggests that the differences between the unknown taxon and *O. mediterranea* are small (Wildish et al. 2012; Pavesi et al. 2014). Thus for the mitochondrial gene, COI, the difference for K2P = 2% with a divergence time of 0.83 ± 1.5 MYA (Pavesi et al. 2014). The low K2P% would not reach species level difference (assumed to be K2P = 3% in Amphipoda, Radulovicí et al. 2009; 4% in Wildish et al. 2012). It is hypothesized that the unknown taxon was formed from an *O. mediterranea* population which found itself in a secondary ecotope (driftwood) where a few, or single pleiotropic, nuclear gene mutations occurred which resulted in slower growth and sexualisation occurring at an earlier moult. Therefore, the unknown taxon would follow life within driftwood and be unable to breed with *O. mediterranea* because of size and habitat differences. If this mutant form arose recently (in geological time), as the COI divergence time suggests, the magnitude of K2P differences may be an inapplicable measure of species or genus level difference. Clearly further genetic evidence to test this hypothesis is required: such as a search for the hypothesized nuclear gene(s) controlling neotenous dwarfism.

Pragmatically, and in the absence of conclusive molecular data, it is considered prudent to remove the unknown taxon from *Orchestia*, a supralittoral wrack generalist genus and create a new driftwood specialist genus: *Neotenorchestia* for the unknown taxon. Finding adult males and females of the new genus is needed to complete the description and diagnosis of the new taxon.

Inclusive of the taxonomic actions taken above brings the total genera of driftwood talitrids to three: *Macarorchestia* Stock 1989, “Platrochestia”* chathamensis* Bousfield 1982 and *Neotenorchestia* gen. n. For the latter genus only one species is known and with the transfer of *microphthalma* Amanieu & Salvat, 1963 from *Orchestia* brings the species belonging to *Macarorchestia* to a total of 5. Thus the known driftwood specialist talitrids total to date is 7 species.

The scarce locality records for each species documented here suggest either rareness and/or that they are difficult to find on shores of the northeast Atlantic and Mediterranean seas. Further evidence for this is that 2 of the 6 driftwood taxa dealt with here are known only from the type locality and the rest from only a few locations. A problem for future discoveries of driftwood talitrids is that the habitats are fast being destroyed by human activities. Documented examples include the destruction of the type locations for two species as mentioned above.

Both *Macarorchestia* and *Neotenorchestia* gen. n. probably originated from ancestors that were larger and faster growing. The evolutionary process in these genera involves reductions in metabolic and growth rates as well as sexualization occurring at an earlier moult number (neotenous dwarfism). A recent, common ancestor gave rise to modern *O. mediterranea* and *N. kenwildishi* gen. n., sp. n., which is consistent with morphological (relative growth) and molecular genetic studies (Wildish et al. 2012; Pavesi et al. 2014). The common ancestor of *Macarorchestia* is unknown, but within the genus of two lineages defined genetically (Wildish et al. 2012):

1. roffensis --- martini
2. remyi --- pavesiae sp. n.

Evolution involves further neotenous dwarfism. Thus taxa to the left are larger and plesiomorphic, whereas those to the right are smaller and apomorphic. Neotenous dwarfism of this kind in driftwood talitrids poses a special challenge to taxonomy because many of the slope values between pairs of species are isometric. In these cases only regression constants, or plots, can be used to separate two species populations. Isometric relative growth is rare.

| Species | Lmnd lacinia mobilis dentition | Numbers of setae in tufts on male propodus of P7 | Number of tufts on posterior side of male propodus of P7 | Mt DNA COI** |
|---------|--------------------------------|-----------------------------------------------|------------------------------------------------|-------------|
| M. microphthalma | 4 | Distal 5 | Anterodistal 0 | 4 | 4 | Yes |
| M. roffensis | 4 | 2 | 0 | 0 | Yes |
| M. martini | 5* | ? | ? | ? | Yes |
| M. remyi | 4-5 | 6 | 2 | 3 | Yes |
| M. pavesiae sp. n. | 4 | 6 | 2 | 3 | Yes |

Table 3. Summary of morphological and molecular data available for species of *Macarorchestia*. * Stock (1989), ** Wildish et al. (2012).
within the Amphipoda and where it does occur ratios cannot be used to express the relative growth differences and recourse to regression predictions appears to be the only way to handle the differences due to neotenous dwarfism. Permanent slides of limb and mouth parts prepared by Sara LeCroy (Gulf Coast Research Lab, University of Southern Mississippi) of four species of *Macaroorchestia* identified possible, taxonomically important, morphological differences between groups 1 and 2 as defined in the preceding paragraph. Thus the left mandible lacinia mobilis in *Macaroorchestia* appeared to be predominantly 4-dentate (Table 3). In an immature male *M. roffensis* the teeth were lateral to the viewing plane and consequently easy to count. In all other preparations, including temporary ones, the teeth were dorsal to the viewing plane (“end on”) making it necessary to focus up and down to see the teeth. An adult male of *M. remyi* appeared to be 5-dentate, whereas an immature female of this species was 4-dentate. Intraspecific variation of left mandible lacinia mobilis dentition has been recognized in other talitrids (Wildish and LeCroy 2014) and this may be the case in *Macaroorchestia*, which renders this character of dubious value in taxonomic discrimination. Further studies are needed to resolve this point. The presence of long, simple, fine setae on the propodus of peraeopod 7 (“comb” setae) proved to be useful in distinguishing species of *Macaroorchestia* (Fig. 3). Thus males of *M. microphalma* had groups of 6 tufts: one near the first insertion of bifid-tipped, robust spines on the anterior side of peraeopod 7 (5 setae), distal (6 setae of 192 µm), anterodistal (0 setae), then 4 setal tufts on the posterior side of the peraeopod at the first insertion of bifid-tipped, robust setae (5 setae), second (5 setae), third (5 setae) and fourth (4 setae). Setal length was approximately the same at each insertion, varying from 156 to 168 µm, except for the most proximal where it was 120 µm. Males of *M. remyi* and *M. pavesiae* sp. n., also had groups of 6 tufts on the propodus of peraeopod 7, but differed from *M. microphalma* in having a single tuft at anterodistal position (2 setae) and only 3 tufts on the posterior propodus. A subadult male *M. roffensis* had a single tuft (2 setae, length 70 µm). This character is sexually dimorphic in all species of *Macaroorchestia* and females have only two tufts at distal and anterodistal positions on the propodus of peraeopod 7 (each of 3-6 setae), which are of smaller setal length than in males. The number of setae in each tuft is growth dependant, so the number of setae per tuft cannot be used as a definitive taxonomic character.

In considering current data of Table 3 and assuming that the left mandible lacinia mobilis dentition is not taxonomically useful, it is possible to propose a subgeneric split based on Atlantic versus Mediterranean coastal ranges of *Macaroorchestia*. This is clearly premature because:

- of missing data as indicated by question marks in Table 3.
- The left mandible lacinia mobilis is 5 dentate according to Stock (1989), rather than 4 dentate, as would be the case if it were close to *M. roffensis* as the molecular data indicates (see Table 5 in Wildish et al. 2012). If intraspecific variation in dentition (4 or 5 dentate) is common this might explain this apparent anomaly.

Further molecular and morphological studies are needed to resolve the subgeneric status of *Macaroorchestia*.

**Acknowledgments**

Thanks to Sara E. LeCroy for preparing permanent slides of *Macaroorchestia* and the unknown taxon, Dr. Laura Pavesi and Kenneth J. Wildish for help in sampling and Dr. Dirk Platvoet (Zoological Museum, Amsterdam) and Miranda Lowe (Natural History Museum, London) for loaning type and other *Macaroorchestia* material. I thank Dr. H. Morino and Mr. J. Valentine for improving earlier versions.

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Checklist of freshwater symbiotic temnocephalans (Platyhelminthes, Rhabditophora, Temnocephalida) from the Neotropics

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Abstract

Based on published records and original data derived from our research, we have generated a checklist of symbiotic temnocephalan fauna from 57 taxa of freshwater invertebrate and vertebrate hosts from 16 families included in four classes from the Neotropics. The checklist contains 38 nominal species from 3 genera belonging to the Temnocephalida families Diceratocephalidae, Didymorchidae and Temnocephalidae. All taxa (35) of the genus Temnocephala are endemic to the Neotropics and 14 (40%) are considered micro-endemic (i.e. only one record each from a single locality). While only one species and one variety of Didymorchis are known from the Neotropics; there are also two putative undescribed species of this genus. Only Diceratocephala boschmai (Diceratocephalidae) is reported as an introduced species from Uruguay. Host specificity to a particular group of invertebrates and vertebrates is the first evidence for the ecological and evolutionary associations that will be analyzed in future studies.

Key Words

Temnocephala
Didymorchis
Diceratocephala
Malacostraca
Insecta
Gastropoda
Chelonia
taxonomy
inventory

Introduction

Temnocephalida (Platyhelminthes, Rhabditophora) is the most diverse group of symbiotic turbellarians typically associated with crustaceans, with 122 valid species and 24 genera described in the world (Tyler et al. 2006–2012). Recently, Temnocephalida was confirmed as a monophyletic group included in Lymnophloplanida, which in turn makes up part of the Dalytyphloplanida clade, a major group of Rhabdocoela (Van Steenkiste et al. 2013). Within the Temnocephalida, the family Temnocephalidae Monticelli, 1899, is the most diverse, distributed in the Australian region with high species richness, but low host diversity, and in the Neotropics with an apparently lower number of temnocephalan species, but a greater diversity of host taxa (Damborenea and Brusa 2009, Sewell 2013). In fact, in the Neotropics, 32 species belonging to the genus Temnocephala and four taxa belonging to Didymorchis, endemic to this region and associated with crustaceans, mollusks, insects and chelonians have been described (Damborenea and Cannon 2001b, Garcés et al. 2013 and cited therein).

The inventory work of the temnocephalan fauna in the Neotropics began in the 18th century, when the first species of Temnocephalida was described, Temnocephala chilensis (Moquin-Tandon 1846), associated with anomuran crabs, Aegla laevis (Latreille), from Chile (Damborenea and Cannon 2001a). Since then, more than 50 studies have been published regarding aspects of the temnocephalan fauna in the Neotropics, including descriptions of new species, analyses of temnocephalan symbiotic community structure of particular host species, and studies with phylogenetic and biogeographic inferences (e.g. Damborenea 1998, Volonterio 2007a, 2014).
Garcés et al. 2013). However, in many cases, information about the reported biodiversity in particular geographical locations of these rhabdocoel turbellarians is scattered among myriad bibliographic sources and difficult to access. Therefore, attempts to generate inventories and compile information are highly valuable for understanding the global diversity of freshwater flatworms (Schockaert et al. 2008). The main objectives of this paper are to compile all the available published accounts on the symbiotic freshwater temnocephalans from the Neotropics and to incorporate new data derived from our own work of the last few years to construct a checklist of symbiont-host associations.

Material and methods

Bibliographic search

All the published records on Neotropical temnocephalan species reported from Malacostraca (Decapoda), Gastropoda (Caenogastropoda), Insecta (Hemiptera, Megaloptera, Plecoptera and Trichoptera) and Reptilia (Testudines) strictly in freshwater systems were compiled. Databases such as Biological Abstracts, Biological and Agricultural Index Plus and Scopus, Google Scholar, Helminthological Abstracts, ISI Web of Knowledge, Turbellarian Taxonomic Database and Zoological Record were used to ensure that we retrieved all available information; the bibliographic search was undertaken up to June, 2014. We considered all the studies whose datasets provide taxonomic information regarding the Neotropical temnocephalan taxa, even those found in a single individual host. Papers containing compiled records of Neotropical temnocephalans that require taxonomic revisions due to problems were indicated (e.g. Vianna and Melo 2002). The host species names were used according with IUCN (2014), Tree of Life Web Project (Maddison and Schulz 2007), and with specific bibliography for Gastropoda (Cowie and Thiengo 2003) and Malacostraca (Melo 2003, De Grave and Ashelb 2013). Furthermore, a species accumulative curve (Dove and Cribb 2006) was obtained to evaluate the extent of the Neotropical symbiotic temnocephalan inventory.

Survey work

Original data from our own studies of the last few years were included. A total of 11 taxa of decapod crustaceans of five families was examined for Argentine and Mexican temnocephalans. Furthermore, two species of Chelonia and one of Gastropoda from Argentina also were examined. Decapod crustaceans were collected with seine nets in one locality of central Mexico (Table 1). The collected decapod crustaceans were kept alive and examined for temnocephalans no more than 4 h after their capture. Decapod crustaceans were sacrificed and immediately examined for temnocephalans; external (e.g. carapace and claw surface) and internal structures (e.g. branchial cavity) were analyzed separately in Petri dishes with 0.65% saline solution, under a stereomicroscope. Gills from each decapod were also obtained and placed in tap water to search for temnocephalans. In the case of mollusk hosts, their mantle cavity was opened after sacrificing. Temnocephalan collections from live turtles were carried out by the catch-and-release method (e.g. FAO 2012); therefore, the live turtles were identified directly in the field (L. Alcalde, personal communication). Temnocephalans were fixed with hot (steaming) 4% formalin or hot (steaming) distilled water. In some cases, specimens from the same host and with the same external aspect were fixed in 100% ethanol in the field for future molecular studies. All temnocephalans were processed following standard procedures (Sewell 2013). Species identification was achieved using special literature, and voucher specimens of some temnocephalans were deposited at the Colección Helmintológica of Museo de La Plata, Argentina (MLP-He) and the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico, as follows: symbionts of Malacostraca (CNHE: 9276-9277; MLP-He: 6148-6155, 6218, 6641, 6763, 6768, 6770, 7153); symbionts of Gastropoda (MLP-He: 3050-3052, 6622, 6764-6767, 6769) and symbionts of Chelonia (MLP-He: 6642-6643).

Results

In total, 60 papers have been published establishing host and locality records of the freshwater temnocephalan fauna in the Neotropics. The analysis of all available information (bibliographic and new original data) allowed us to establish a list of 38 symbiotic temnocephalan taxa in invertebrates and vertebrates in the Neotropical region, which are contained in four groups of hosts. Malacostraca (Decapoda): 4 taxa of Didymorchis associated with 3 taxa of crabs, 17 species of Temnocephala associated with 32 taxa of decapod crustaceans and only one species of Diceratocephala associated with one species of decapod crustacean; Gastropoda (Caenogastropoda): 5 species of Temnocephala associated with 5 taxa of freshwater snail hosts; Insecta: 1, 2, 2 and 1 taxa of Temnocephala associated with 1, 5, 3 and 1 taxa hosts of Trichoptera, Hemiptera, Megaloptera and Plecoptera, respectively; Chelonia (Testudines): 4 taxa of Temnocephala associated with 7 species of freshwater turtle hosts.

The results of this study are presented in the Table 1 which shows the symbiont-host list, where temnocephalans are organized by taxonomic groups and ordered alphabetically by family name. Then species within each family are listed alphabetically followed by authority name and date. The next category is the host species in which the temnocephals were found, followed by the locality, and the bibliographic reference from which the information was obtained, except for those records established in the present work. In the temnocephalan species found in more than one host species, the latter are listed alphabetically, and host
### Table 1: Freshwater symbiotic Temnocephalida list from Neotropics. AR = Argentina; BR = Brazil; CL = Chile; CO = Colombia; CR = Costa Rica; MX = Mexico; PY = Paraguay; PE = Peru; UY = Uruguay. NR = Coordinates not reported. *Coordinates not presented in the original work, but obtained for this study using Google Earth (https://earth.google.com/).

| Symbiotic species | Host | Locality | Geographical coordinates | Reference |
|-------------------|------|----------|--------------------------|-----------|
| Temnocephalidae Blanchard, 1849 | Didymorchis haswelli | Aiguá, Lavalleja, UY 34°12'08''S; 54°46'16''W | Mañé-Garzon (1960) |
| | Didymorchis haswelli var. australis | Dioni, 1972 | Nahuel Huapi National Park, Río Negro, AR 41°02'35''S; 71°28'06''W | Dioni (1972) |
| | Didymorchis sp. 1 | Damborenea and Cannon (2001a) | Aegla neuquensis, Nahuel Huapi Lake, Bariloche, Río Negro, AR 41°07'54.3''S; 71°19'51.5''W | Damborenea and Cannon (2001a) |
| | Didymorchis sp. 2 | Damborenea et al. (2007) | Aegla neuquensis, Nahuel Huapi Lake, Bariloche, Río Negro, AR 41°07'54.3''S; 71°19'51.5''W | Damborenea et al. (2007) |
| | Didymorchis sp. 3 | Own findings | Aegla neuquensis, Nahuel Huapi Lake, Bariloche, Río Negro, AR 41°07'54.3''S; 71°19'51.5''W | Own findings |
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| Symbiotic species | Host                     | Locality                                      | Geographical coordinates | Reference                  |
|-------------------|--------------------------|-----------------------------------------------|--------------------------|----------------------------|
| Acanthochelys radiolata | BR                       | Viamão, Rio Grande do Sul, BR                | 30°04'56.64''S, 51°01'11.81''W | Monticelli (1889)          |
| Acanthochelys spiculiferum | BR                       | Angra dos Reis, Rio de Janeiro, BR             | 23°01'13.9''S, 44°19'55.7''W | Pereira and Cuocolo (1940) |
| Hydromedusa maximili | BR                       | Juiz de Fora, Minas Gerais, BR                 | 21°41'20.5''S, 43°26'40.4''W | Novelli et al. (2009)      |
| Hydromedusa tectifera  | BR                       | Recife, Rio de Janeiro, BR                      | 23°28'16.2''S, 44°27'04.7''W | Pereira and Cuocolo (1941) |
| Temnocephala brevicornis Monticelli, 1889 | Narra   | Terezaopolis, Rio de Janeiro, BR               | 22°2'50.06''S, 42°5'53.2''W | Pereira and Cuocolo (1940) |
|                   | BR                       | Vicosa, Minas Gerais, BR                        | 20°45'17.52''S, 42°52'48.8''W | Pereira and Cuocolo (1940) |
|                   | Narra   | Carnaval Stream, City Bell, Buenos Aires, AR   | 34°53'46.0''S, 58°05'39.0''W | Own findings              |
|                   | Narra   | Chubichamini Stream, Magdalena, Buenos Aires, AR | 35°03'55.3''S, 57°37'37.2''W | Own findings              |
|                   | Narra   | Valle Edén, Tacuarembo, UV                      | 31°49'11.5''S, 56°10'37.8''W | Dioni (1976a)             |
|                   | Narra   | Villoldo Stream, Magdalena, Buenos Aires, AR   | 35°15'56.0''S, 57°15'32.2''W | Bussa and Damborenea (2000) |
|                   | Narra   | Varginha, São Paulo, BR                         | 20°45'11.8''S, 42°52'48.8''W | Pereira and Cuocolo (1940) |
|                   | Narra   | Villoldo Stream, Magdalena, Buenos Aires, AR   | 35°15'56.0''S, 57°15'32.2''W | Bussa and Damborenea (2000) |
|                   | Narra   | Rezende, Rio de Janeiro, BR                      | 21°41'20.5''S, 43°26'40.4''W | Novelli et al. (2009)      |
|                   | Narra   | Juiz de Fora, Minas Gerais, BR                  | 21°41'20.5''S, 43°26'40.4''W | Novelli et al. (2009)      |
|                   | Narra   | Fortaleza, Rio de Janeiro, BR                    | 23°28'16.2''S, 44°27'04.7''W | Pereira and Cuocolo (1941) |
|                   | Narra   | Varghina, São Paulo, BR                          | 20°45'11.8''S, 42°52'48.8''W | Pereira and Cuocolo (1940) |
|                   | Narra   | Rezende, Rio de Janeiro, BR                      | 23°28'16.2''S, 44°27'04.7''W | Pereira and Cuocolo (1941) |
|                   | Narra   | Terezaopolis, Rio de Janeiro, BR                 | 22°2'50.06''S, 42°5'53.2''W | Pereira and Cuocolo (1940) |
|                   | Narra   | Vicosa, Minas Gerais, BR                           | 20°45'17.52''S, 42°52'48.8''W | Pereira and Cuocolo (1940) |
|                   | Narra   | Carnaval Stream, City Bell, Buenos Aires, AR   | 34°53'46.0''S, 58°05'39.0''W | Own findings              |
|                   | Narra   | Chubichamini Stream, Magdalena, Buenos Aires, AR | 35°03'55.3''S, 57°37'37.2''W | Own findings              |
|                   | Narra   | Valle Edén, Tacuarembo, UV                      | 31°49'11.5''S, 56°10'37.8''W | Dioni (1976a)             |
|                   | Narra   | Villoldo Stream, Magdalena, Buenos Aires, AR   | 35°15'56.0''S, 57°15'32.2''W | Bussa and Damborenea (2000) |
|                   | Narra   | Varginha, São Paulo, BR                          | 20°45'11.8''S, 42°52'48.8''W | Pereira and Cuocolo (1940) |

Remarks: Caballero y Caballero and Cerecero (1951) mentioned Temnocephala brevicornis being a comensal of Pseudothelphusa sp. (Crustacea) from El Salvador and Venezuela; however, this association has to be considered with caution because of the probable misidentification of the specimens.
| Symbiotic species | Host | Locality | Geographical coordinates | Reference |
|-------------------|------|----------|--------------------------|------------|
| **Parastacus sp.** | Meliquina River, flows into Meliquina Lake, Neuquén, AR | 40°23.007’S; 71°15.916’W* | Own findings |
| | Nahuel Huapi Lake, Río Negro, AR | 41°02.25’S; 71°28.06’W* | Dioni (1967a), Dioni (1972) |
| | Near Santiago city, CL | 33°28.08’S; 70°38.31’W* | Moquín-Tandon (1864) |
| | Parana River, Rosario, Santa Fe, AR | 32°57.03’S; 60°7.07’W* | Dioni (1967c)(*) |
| | Uspallata, Mendoza, AR | 32°59.14’S; 69°2.20’W* | Dioni (1967c) |
| | Nahuel Huapi Lake, Río Negro, AR | 41°02.25’S; 71°28.06’W* | Damborenea (1992) |
| | Tumbos, Táchira, CL | NR | Wickie (1905) |
| | Limay River, Rincón de Creide, Neuquén, AR | 40°47.14’S; 71°07.08’W* | Own findings |

**Remarks.** Taking into account the known distribution of *T. chilensis*, the citations marked with (+) are considered doubtful.

- *Temnocephala colombiensis* Garncés, Puerta, Tabares, Laviñ and Velásquez, 2013
  - Pomacea sp. San José del Núi Ponds, San Roque, Antioquia, CO 6°29’51”N; 74°50’28”W* | Garcés et al. 2013 |
  - *Pseudotelmaphora tristani* Rincón de Osa, Punta Arenas, CR 9°58.00’S; 84°50.00’W* | Lamotte-Agundez (1974) |

- *Temnocephala costarricensis* Lamotte, 1974
  - Belostoma dentatum State of Minas Gerais, BR NR | Amato and Amato (2005) |
  - Belostoma dilatum Taruma Lake, Rio Grande do Sul, BR 20°04’04”S; 44°00’00”W | Amato and Amato (2005) |
  - Belostoma elegans Stream and canal, Arrozeira, Eldorado do Sul, Rio Grande do Sul, BR 30°13’36”S; 51°22’42”W | Amato and Amato (2005) |
  - Belostoma testacopallidum Retiro das Pedras, Itanhaém, Minas Gerais, BR 20°04’04”S; 44°00’00”W | Amato and Amato (2005) |

- *Temnocephala curvicirri* Amato and Amato, 2005
  - *Belostoma cummingsi* Otamendi, Paraná Delta, Buenos Aires, AR 29°15’00”S; 50°15’45”W* | Amato et al. (2003) |
  - *Palaemon argentinus* Los Talas, Barrio, Buenos Aires, AR 34°57’18”S; 57°49’49”W | Damborenea (1992) |

- *Temnocephala digitata* Monticelli, 1902
  - *Belostoma digitata* Monticelli, 1902
  - Palaemon canaliculata Freshwater bodies from Buenos Aires, in communication with Río de la Plata, AR NR | Monticelli (1902) |
  - Palaemon argentinus Los Talas, Barrio, Buenos Aires, AR 34°57’18”S; 57°49’49”W | Damborenea (1992) |

- *Temnocephala decarolii* Ponce de León, 1989
  - *Aegla serrana* Creek and a small reservoir in the Utopia II Farm, Cambará do Sul, RS, and Río Grande do Sul, BR 29°15’00”S; 50°15’45”W* | Ponce de León (1989) |

- *Temnocephala haswelli* Ponce de León, 1989
  - *Belostoma cummingsi* Otamendi, Paraná Delta, Buenos Aires, AR 29°15’00”S; 50°15’45”W* | Amato et al. (2003) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Temnocephala iheringi* Haswell, 1893
  - *Belostoma cummingsi* Otamendi, Paraná Delta, Buenos Aires, AR 29°15’00”S; 50°15’45”W* | Seixas et al. (2010c) |

- *Temnocephala oswaldii* Volontéo, 2010
  - *Hydromedusa testacopallidum* Colorado Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2010) |

- *Temnocephala cyanoglandula* Amato and Amato, 2003
  - *Belostoma cummingsi* Otamendi, Paraná Delta, Buenos Aires, AR 29°15’00”S; 50°15’45”W* | Amato et al. (2003) |

- *Temnocephala digitata* Monticelli, 1902
  - *Palaemon canaliculata* La Virgen Stream, UY 34°25’15”S; 56°24’34”W | Dioni (1966) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Asolene platae* Paso del Horro (Route 6, km 5), Sauce Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2007a) |

- *Temnocephala cyanoglandula* Amato and Amato, 2003
  - *Palaemon canaliculata* Malabrigo Stream, tributary of the Río San Javier, Santa Fe, AR 30°06’04”S; 57°37’20”W | Damborenea (2006) |

- *Temnocephala cyanoglandula* Amato and Amato, 2003
  - *Palaemon canaliculata* Malabrigo Stream, tributary of the Río San Javier, Santa Fe, AR 30°06’04”S; 57°37’20”W | Damborenea (2006) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Pomacea canaliculata* San José del Núi Ponds, San Roque, Antioquia, CO 6°29’51”N; 74°50’28”W* | Garcés et al. 2013 |

- *Aegla serrana* Creek and a small reservoir in the Utopia II Farm, Cambará do Sul, RS, and Río Grande do Sul, BR 29°15’00”S; 50°15’45”W* | Amato et al. (2003) |

- *Pomacea canaliculata* Malabrigo Stream, tributary of the Río San Javier, Santa Fe, AR 30°06’04”S; 57°37’20”W | Damborenea (2006) |

- *Asolene platae* Madrejón Don Felipe, Santa Fe, AR NR | Damborenea (1992) |

- *Pomacea canaliculata* Paso del Horro (Route 6, km 5), Sauce Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2007a) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Pomacea canaliculata* Paso del Horro (Route 6, km 5), Sauce Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2007a) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Pomacea canaliculata* Paso del Horro (Route 6, km 5), Sauce Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2007a) |

- *Asolene platae* Parque Marihna de Brasil, Porto Alegre, BR 30°03’34”S; 51°13’49”W | Seixas et al. (2010c) |

- *Pomacea canaliculata* Paso del Horro (Route 6, km 5), Sauce Stream, Canelones, UY 34°38’00”S; 56°06’00”W | Volontéo (2007a) |
| Symbiotic species | Host | Locality | Geographical coordinates | Reference |
|-------------------|------|----------|--------------------------|-----------|
| Temporal body water, Route 14, Entre Ríos, AR | | 33°21'11''S; 58°48'41''W | Own findings |
| Bird Observatory, Rocha, UY | | 33°54'S; 53°40'W | Volontério (2007a) |
| Bridge over Andreaoni channel, Rocha, UY | | 33°54'S; 53°40'W | Volontério (2007a) |
| Bridge over cañada, Rocha, UY | | 33°51'S; 53°55'W | Volontério (2007a) |
| Bridge over the Averías Stream, Rocha, UY | | 33°53'S; 53°51'W | Volontério (2007a) |
| Cachoeira River, Barragem do Cerrito, Barra do Ouro, Maquiné, Río Grande do Sul, BR | | 29°34'15''S; 50°16'51''W | Seixas et al. (2010a) |
| Cañada 1, Soriano, UY | | 33°12'S; 57°27'W | Volontério (2007a) |
| Cañada del Ceibo, Colonia, UY | | 34°40'S; 56°03'W | Volontério (2007a) |
| Channels around rice, Arrozeira, Eldorado do Sul, Río Grande do Sul, BR | | 30°13'56''S; 51°22'42''W | Seixas et al. (2010a) |
| Chapasolli Stream, Buenos Aires, AR | | 36°48'53''S; 59°08'05''W | Martin et al. (2005) |
| Chasico Stream, near Los Chilenos Lake, Buenos Aires, AR | | 38°09'59''S; 62°36'02''W | Martin et al. (2005) |
| Colonia city, near the wall, Colonia, UY | | 34°28'S; 57°50'W | Volontério (2007a) |
| Dría Flera Stream, Buenos Aires, AR | | 34°50'57''S; 57°52'20''W | Damborenea (1992) |
| El Fuerte reservoir at Tandil city, Buenos Aires, AR | | 37°25'41''S; 59°07'45''W | Martin et al. (2005) |
| El Pescado stream, near the Río de la Plata estuary, Buenos Aires, AR | | 34°57'S; 57°46'W | Damborenea et al. (2006) |
| Kásel Hainanc reservoir, Route 2, Buenos Aires, AR | | 36°47'36''S; 57°46'29''W | Martin et al. (2005) |
| Fazenda Sossego, Santa Vitória do Palmar, Río Grande do Sul, BR | | 33°16'13''S; 53°26'28''W | Seixas et al. (2010a) |
| India Muerta Stream, Rocha, UY | | 33°46'S; 54°08'W | Volontério (2007a) |
| Interbalnearia Route, Canelones, UY | | NR | Flecher and Ponce de León (1983) |
| Italia Avenue, Canelones, UY | | NR | González et al. (1987) |
| Juncaal Stream, Flores, UY | | 33°29'S; 56°58'W | Volontério (2007a) |
| La Lancha Stream, Soriano, UY | | 33°37'S; 57°58'W | Volontério (2007a) |
| Languayú Stream, Buenos Aires, AR | | 36°40'24''S; 58°26'42''W | Martin et al. (2005) |
| Los Chilenos lake, Fishing Club Torquati, Buenos Aires, AR | | 38°02'37''S; 62°31'01''W | Martin et al. (2005) |
| Los Padres lake, Buenos Aires, AR | | 37°59'06''S; 57°43'54''W | Martin et al. (2005) |
| Los Talas, Berisso, Buenos Aires, AR | | 34°55'00''S; 57°49'57''W | Damborenea (1992) |
| Los Talas lagoon, near the Río de la Plata estuary, Buenos Aires, AR | | 34°57'S; 57°46'W | Damborenea et al. (2006) |
| Martín García Island, Río de la Plata, AR | | 34°10'45''S; 58°15'08''W | Own findings |
| Miguellín Stream, Punta Lara, Ensenada, Buenos Aires, AR | | 34°48'00''S; 57°59'22''W | Damborenea (1992) |
| Paraná Medio, AR | | 30°02'23''S; 51°25'49''W | Di Persia and Radici de Cura (1973) |
| Paso de la Cruz, tributary of Bequelló Stream, Soriano, UY | | 33°13'S; 57°55'W | Volontério (2007a) |
| Paso de Las Piedras reservoir, Buenos Aires, AR | | 38°24'32''S; 61°43'37''W | Martin et al. (2005) |
| Ponta do Ceroula, Barra do Ribeiro, Río Grande do Sul, BR | | 30°15'51'S; 51°16'49.55''W | Seixas et al. (2010a) |
| Praia Florida, Guaiabi, Río Grande do Sul, BR | | 30°15'54''S; 51°12'25''W | Seixas et al. (2010a) |
| Punta Indio, Buenos Aires, AR | | 35°19'S; 57°13'W | Damborenea (1992) |
| Jacuí River, at Ilha da Pintada, Porto Alegre, Río Grande do Sul, BR | | 30°22'33''S; 51°25'49''W | Seixas et al. (2010a) |
| Route 4 near Los Cerrillos, Entre Ríos, AR | | 31°21'11''S; 58°6'16''W | Own findings |
| San Pedro Stream, Colonia, UY | | 34°18'S; 57°52'W | Volontério (2007a) |
| Sarandi Chico Stream, Flores, UY | | 33°37'S; 56°51'W | Volontério (2007a) |
| Sarandi del Consejo Stream, Rocha, UY | | 34°15'S; 53°59'W | Volontério (2007a) |
| Symbiotic species | Host | Locality | Geographical coordinates | Reference |
|-------------------|------|----------|--------------------------|-----------|
| *Sauce Stream, Canelones, UY* | *Sauce Stream, Canelones, UY* | 34°38'S; 56°3'W | Volonterio (2007a) |
| *Sava Clube, Guaiuba Lake, Porto Alegre, Rio Grande do Sul, BR* | *Sava Clube, Guaiuba Lake, Porto Alegre, Rio Grande do Sul, BR* | 30°06'S; 51°15'57.5"W | Seixas et al. (2010a) |
| *Sevedra Park, La Plata, Buenos Aires, AR* | *Sevedra Park, La Plata, Buenos Aires, AR* | 34°55.2'.1"S; 57°54.31.58"W | Own findings |
| *Stream 1, Rocha, UY* | *Stream 1, Rocha, UY* | 33°26'S; 53°52'W | Volonterio (2007a) |
| *Stream 2, Rocha, UY* | *Stream 2, Rocha, UY* | 33°24'S; 53°51'W | Volonterio (2007a) |
| *Stream near Route 11, Magdalena, AR* | *Stream near Route 11, Magdalena, AR* | 34°57'.53"S; 57°40'.91"W | Own findings |
| *Zapata Stream, Magdalena, AR* | *Zapata Stream, Magdalena, AR* | 34°59'.18"S; 57°43'00"W | Damborenea (1992) |
| *Guaicurus, Mato Grosso do Sul, BR* | *Guaicurus, Mato Grosso do Sul, BR* | 20°51'S; 56°45'W | Pereira and Cuocolo (1941) |
| *Salobra, Mato Grosso do Sul, BR* | *Salobra, Mato Grosso do Sul, BR* | 20°10'S; 56°31'W | Pereira and Cuocolo (1941) |
| *Own findings* | *Own findings* | | |
| *Stream 1, Rocha, UY* | *Stream 1, Rocha, UY* | 33°26'S; 53°52'W | Volonterio (2007a) |
| *Sevedra Park, La Plata, Buenos Aires, AR* | *Sevedra Park, La Plata, Buenos Aires, AR* | 34°55.2'.1"S; 57°54.31.58"W | Own findings |
| *Stream 2, Rocha, UY* | *Stream 2, Rocha, UY* | 33°24'S; 53°51'W | Volonterio (2007a) |
| *Stream near Route 11, Magdalena, AR* | *Stream near Route 11, Magdalena, AR* | 34°57'.53"S; 57°40'.91"W | Own findings |
| *Zapata Stream, Magdalena, AR* | *Zapata Stream, Magdalena, AR* | 34°59'.18"S; 57°43'00"W | Damborenea (1992) |
| *Guaicurus, Mato Grosso do Sul, BR* | *Guaicurus, Mato Grosso do Sul, BR* | 20°51'S; 56°45'W | Pereira and Cuocolo (1941) |
| *Salobra, Mato Grosso do Sul, BR* | *Salobra, Mato Grosso do Sul, BR* | 20°10'S; 56°31'W | Pereira and Cuocolo (1941) |

**Remarks.**

Dioni (1972) mentioned *T. mexicana* on *Aegla sp.* and *Parastacus sp.* from Nahuel Huapi Lake, Rio Negro, AR 41°02.35'S; 71°28.06"W.
| Symbiotic species                  | Host                      | Locality                         | Geographical coordinates | Reference                  |
|-----------------------------------|---------------------------|----------------------------------|--------------------------|---------------------------|
| Temnocephala microdactyla Monticelli, 1903 | Dilocarcinus pagei         | Guadalupe Lagoon, Santa Fe, AR   | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    |                           | Mato Grosso, BR                   | NR                       | Monticelli (1903)         |
|                                    |                           | Piedras Blancas, Guadalupe Lagoon, Santa Fe, AR | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    |                           | NR                               | NR                       | Monticelli (1903)         |
|                                    | Sylvicarcinus australis   | Piedras Blancas, Guadalupe Lagoon, Santa Fe, AR | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    | Sylvicarcinus pictus      | Bodóquera, Mato Grosso, BR        | 20°32'9''S; 56°4'25''W   | Pereira and Cucó (1941)   |
| Temnocephala minutocirrus Amato, Seixas and Amato, 2007 | Cryptocricos granulosus   | Forqueta Stream, Barra do Ouro, Maquéní, Río Grande do Sul, BR | 29°3'21''S; 50°14'7''W   | Amato et al. (2007)       |
|                                    |                           | Garapi Stream, Barra do Ouro, Maquéní, Río Grande do Sul, BR | 29°3'23''S; 50°14'38''W  | Amato et al. (2007)       |
|                                    |                           | Río da Divisa, São José dos Ausentes, Río Grande do Sul, BR | 28°3'17''S; 04°57'46''W  | Amato et al. (2007)       |
| Temnocephala peruensis Ibáñez Herrezn and Jarab, 2003 | Hypholobocera heinerti    | Berta de Carrasco, Canelones, UY  | 34°52'S; 56°02'W         | Volontério (2010)         |
|                                    |                           | Colorado Stream, Canelones, UY    | 34°38'S; 56°03'W         | Volontério (2010)         |
| Temnocephala pignalberiae Domí, 1967 | Dilocarcinus pagei         | Candeiro stream, Ecological Station, Belvedouro, São Paulo, BR | 20°56'58''S; 48°28'43''W | Domí (2010)               |
|                                    |                           | Fazenda Ypiranga, Poconé, 10 km south of Poconé and 110 km from Cuiabá, Mato Grosso, BR | 16°20'14''S; 56°38'58''W | Amato et al. (2010)       |
|                                    |                           | Guadalupe Lake, Piedras Blancas, Santa Fe, AR | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    |                           | Los Espejos Lagoon, Santa Fe, AR  | NR                       | Domí (1967d)              |
|                                    |                           | Madejón Don Felipe, Santa Fe, AR  | NR                       | Domí (1967d)              |
|                                    |                           | Piedras Blancas, Guadalupe Lagoon, Santa Fe, AR | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    |                           | Madejón Don Felipe, Santa Fe, AR  | NR                       | Domí (1967d)              |
|                                    | Sylvicarcinus australis   | Guadalupe Lake, Santa Fe, AR      | 31°33'46''S; 60°36'20''W | Domí (1967d)              |
|                                    |                           | Madejón Don Felipe, Santa Fe, AR  | NR                       | Domí (1967d)              |
|                                    |                           | Piedras Blancas, Guadalupe Lagoon, Santa Fe, AR | 31°39'S; 60°42'W         | Domí (1967d)              |
|                                    |                           | Trichodactylus panoplus          | 33°13'S; 57°55'W         | Volontério (2007a)        |
|                                    |                           | Trichodactylus sp.               | NR                       | Domí (1967d)              |
| Temnocephala rochensis Ponce de Léon, 1979 | Pomacea canaliculata      | Paracatuí, Entre Rios, AR         | 31°45'55''S; 60°31'25''W | Domí (1967d)              |
|                                    |                           | Bird Observatory (Route 14, km 487), Rocha, UY | 33°54'S; 53°40'W         | Volontério (2007a)        |
|                                    |                           | Cebollati River (Route 15, km 195), UY | 33°14'S; 53°47'W         | Volontério (2007a)        |
|                                    |                           | Channel on the road to São Lorenzo do Sul, BR | 31°20'14''S; 52°03'10''W | Seixas et al. (2010b)     |
|                                    |                           | JuncaÍ Stream, Fazenda da Invernada, BR | 32°2'08'8''S; 53°15'44'6''W | Seixas et al. (2010b)     |
|                                    |                           | Fazenda Sossegó, 30 km west of Santa Vitoria do Palmar, BR | 33°16'13''S; 53°26'28''W | Seixas et al. (2010b)     |
|                                    |                           | Negro Lake, Rocha, UY             | 34°01'S; 53°30'W         | Ponce de León (1979)      |
|                                    |                           | Pond at Fazenda da Invernada, BR  | 32°2'74'1''S; 53°15'14''W | Seixas et al. (2010b)     |
|                                    |                           | Route 15, km 202, Pond, UY        | 33°13'S; 53°48'W         | Volontério (2007a)        |
|                                    |                           | Route 14, km 446, India Muerta stream, UY | 33°46'S; 54°05'W         | Volontério (2007a)        |
|                                    |                           | Route 14, km 469, Bridge on the Averías Stream, UY | 33°53'S; 53°51'W         | Volontério (2007a)        |
|                                    |                           | Sarandi do Correio Stream (Route 9 km 251), UY | 34°15'S; 53°59'W         | Volontério (2007a)        |
|                                    |                           | Side way ditch on the road to Fazenda São João 2, Jaugarão, BR | 32°35'29'7''S; 53°14'04'5''W | Seixas et al. (2010b)     |
|                                    |                           | Side way pond, Interstate Road 116 (km 12), BR | 32°2'21'2''S; 53°17'20''W | Seixas et al. (2010b)     |
|                                    |                           | Route 15, km 195, Stream, Rocha, UY | 33°26'S; 53°52'W         | Volontério (2007a)        |
| Symbiotic species                  | Host                          | Locality                                    | Geographical coordinates | Reference                  |
|-----------------------------------|-------------------------------|---------------------------------------------|--------------------------|----------------------------|
| Temnocephala santafesina Dioni, 1967 | Dilocarcinus pagei            | Los Espejos Lagoon, Santa Fe, AR            | NR                       | Dioni (1967d)              |
|                                   |                               | Madejón Don Felipe, Santa Fe, AR            | NR                       | Dioni (1967d)              |
|                                   |                               | Piedras Blancas, Guadalape Lagoon, Santa Fe, AR | 31°39'S; 60°42'W*             | Damborenea (1992)          |
| Temnocephala sp.                  | Corydalis sp.                 | Cerrado formation municipality, BR          | 19°57'10"S; 40°32'25"W   | Trivinho-Strixino et al. (2012) |
| Temnocephala sp.                  | Protocauliodes sp.            | Cerrado formation municipality, BR          | 19°57'10"S; 40°32'25"W   | Trivinho-Strixino et al. (2012) |
| Temnocephala sp.                  | Kempnyia reticulata           | Rivers of Estación Biológica de Santa Lucia, Santa Teresa, Espirito Santo, BR | 19°57'10"S; 40°32'25"W   | Avelino-Capistrano et al. (2013) |
| Temnocephala sp.                  | Hydromedusa tectifera         | Palma, Encruzilhada do Sul, BR              | 30°34'-30°43'S; 52°30'-52°51'W | Soares et al. (2007)      |
|                                   | Aegla platensis               | Anchorena Stream, Martinez, Buenos Aires, AR | 34°29'20"S; 58°29'53"W*   | Damborenea (1992)          |
|                                   |                               | Lavalleja (Route 8, km 238), UY            | 33°36'S; 54°35'W            | Volontero (2007a)         |
|                                   |                               | Martin García Island, Rio de la Plata, Buenos Aires, AR | 34°10'45"S; 58°15'08"W   | Damborenea et al. (1992)  |
|                                   | Aegla prado                   | Villa Rica, PY                              | 25°46'50"S; 56°26'55"W*   | Dioni (1968)              |
|                                   | Aegla uruguayana              | El Prado Botanic Garden, Montevideo, UY     | 34°49'9"S; 56°12'W           | Volontero (2009b)         |
|                                   |                               | Martin García Island, Rio de la Plata, Buenos Aires, AR | 34°10'45"S; 58°15'08"W   | Damborenea et al. (1997)  |
|                                   |                               | Moller Stream (Route 8, km 238), Lavalleja, UY | 33°36'S; 56°35'W            | Volontero (2006b)        |
|                                   |                               | Olivos, Buenos Aires, AR                   | 33°30'S; 63°10'W*             | Damborenea (1992)          |
|                                   |                               | Paulino Island, Rio de la Plata, Buenos Aires, AR | 34°50'08"S; 57°52'44"W   | Damborenea (1992)          |
|                                   |                               | Lunairejo Stream, Rivera, UY               | 31°15'50"S; 55°50'0"W*       | Dioni (1967)              |
|                                   |                               | De Doll Stream, Route 11, Entre Rios, AR    | 32°18'37"S; 60°25'54"W*    | Own findings             |
|                                   |                               | Tres Islas, Cerro Lago, UY                 | 32°34'0"S; 53°38'0"W*       | Dioni (1967b)             |
|                                   |                               | Prado, Montevideo, UY                      | NR                       | Dioni (1967b)             |
| Temnocephala talicei Dioni, 1967   | Aegla sp.                     | Agua Parada Stream, Maquiné River Basin, Maquiné, Rio Grande do Sul, BR | 29°06'20"S; 50°21'15"W   | Amato et al. (2006)       |
|                                   |                               | Carvão Stream, Maquiné River Basin, Maquiné, Rio Grande do Sul, BR | 29°32'29"S; 50°13'49"W   | Amato et al. (2006)       |
|                                   |                               | Forqueta Stream, Maquiné River Basin, Maquiné, Rio Grande do Sul, BR | 29°32'17"S; 50°14'44"W   | Amato et al. (2006)       |
|                                   |                               | Vale das Tintas head waters of Rio das Antas, Taquari-Antas basin, São Jose dos Ausentes, Rio Grande do Sul, BR | 28°47'00"S; 49°50'53"W   | Amato et al. (2006)       |
| Temnocephala trapeziformis Amato, Amato and Sebás, 2006 | Trichodactylus flavisialis | San Bernardo, São Paulo, BR                 | 22°00'0"S; 49°00'0"W      | Pereira and Cuoccolo (1941) |
| Temnocephala travassosfilho Pereira and Cuoccolo, 1941 | Trichodactylus metopolitanus | San Bernardo, São Paulo, BR                 | 22°00'0"S; 49°00'0"W      | Pereira and Cuoccolo (1941) |
species for which more than one locality was recorded, are listed together. Furthermore, a host-symbiont list (See Appendix 1) is taxonomically and alphabetically organized.

The decapods are the most species-rich host group with temnocephalans (27), followed by the insects (5 taxa) and snails (5 species). Of the 38 taxa of Temnocephalidae listed in this work, all appear to be specific to particular host groups, while at least only one species of the family Diceratocephalidae have successfully associated with hosts after their anthropogenic introduction, i.e. Diceratocephala boschmai. The most widely distributed species are T. axenos, T. chilensis and T. iheringi, which are present in 9 and 10 crab host species and 5 snail host species, along 20, 25 and 49 localities, respectively.

In terms of hosts, Hydromedusa tectifera (a turtle) is the host with the highest temnocephalan species richness with 4 taxa, followed by Aegla neuquensis, A. platensis, Dilocarcinus pagei (decapod crabs) and Pomacea canaliculata (snail), all with 3 species; meanwhile, 49 host taxa show only one record of temnocephalan taxa for one locality.

The species accumulation curve for Neotropic temnocephalans plotted against the total number of species (Figure 1) shows irregular growth over 15 decades of studies in Temnocephalida (each decade divided into two periods of five years). This graph shows that the asymptote has not been reached yet and, if the systematic studies of the group are continued, a significant increase in the number of species in the Neotropical region can be expected. This graphic also reflects two important periods of research. The first shows the initial prospecting for temnocephalan species in the Neotropical region, between 1890 and the beginnings of the 20th century. The second period, beginning around 1970, shows an increase in the research on temnocephalans from different host species, with some stationary periods.

Discussion

The genus Temnocephala is an endemic component of the Neotropical region (Damborenea and Cannon 2001a). At the moment, it includes 35 taxa, of which 14 (40%) are considered microendemic (only one record for locality) (Table 1). In total, 57 host taxa are associated with one or more temnocephalan taxa, which belong to seven orders and 14 families within four classes. It is worth pointing out that each major group of hosts is characterized by a particular assemblage of temnocephalan species, with host specificity at family level. For example, 17 taxa of Temnocephala are associated with three families of freshwater crab hosts (Aeglidae, Pseudothelphusidae and Trichodactylidae), while five Temnocephala species are associated with 8 taxa of freshwater shrimps included in three families (Cambariade, Palaemonidae and Parastaciidae). Information about the natural history of this endemic genus is key to understanding the role of different factors that shaped its diversification patterns across several hydrological basins in the Neotropics and the possible implications of codivergence with host groups (see below) (e.g. Thompson 2005, Martínez-Aquino et al. 2014b).

In this inventory, only Diceratocephala boschmai was detected as an introduced species because of translocation together with their crustacean hosts, the invasive redclaw Cherax quadricarinatus in Uruguay (Volonterio 2009a), due to human activities such as aquaculture and breeding of ornamental species (Lodge et al. 2012, Saoud and Ghanawi 2013). According to several authors, D. boschmai causes a detrimental economic impact because of an aesthetic effect of the eggs on the body surface of the C. quadricarinatus (Herbert 1987, Volonterio 2009a). However, it is more important to mention the detrimental biological and ecological impact of these introduced
species (e.g. Ahyong and Yeo 2007, Larson and Olden 2012). To date, the values of ecological infection parameters (e.g. prevalence and abundance; see Bush et al. 1997) are unknown not only locally but globally. These parameters are required to measure the effect of this symbiotic association – both introduced species, *C. quadricarinatus* and *D. boschmai* – to detect the extent of the spread of *D. boschmai* to other crustacean taxa, especially endemic crabs in their natural ecosystems (Jones and Lester 1993, Chivavaya 2013, du Preez and Smit 2013). Furthermore, the introduced populations of *D. boschmai* in natural hydrological systems in Uruguay represent a serious problem of displacement to the endemic populations of Neotropical *Temnocephala* species because of interspecific competition between symbiotic organisms (Gelder 1999, Sicard et al. 2006, Witte et al. 2008, Tsuchida et al. 2011, Ohtaka et al. 2012). In this context, the data generated in this checklist can be used to support conservation strategies for freshwater biodiversity (Cardoso et al. 2011a, b, Stendera et al. 2012, Collen et al. 2013).

One hundred sixty eight years have passed since the first description and record of a temnocephalan from the Neotropics (Damborenea and Cannon 2001a), and, currently, ±236 records of temnocephalans have been published. However, considering the number of described species and the time passed, it can be stated that most of the diversity of *Temnocephala* remains yet to be described. There is also a significant number of potential hosts that have not been studied with regards to symbiotic temnocephalans. On the other hand, Schoackaert et al. (2008) mentioned that the few species recorded in South America were mostly recorded up to about 1970. Based on the species accumulation curve (Figure 1), this study shows clearly the increase in knowledge about the biodiversity of the temnocephalan fauna in recent times, but based on all of the information compiled for Neotropic temnocephalans, we show the necessity to continue inventory work. The Neotropic temnocephalan fauna contains 31% of Temnocephalida taxa described at the moment, representing 37 taxa allocated to two genera.

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**Figure 2.** Map of Latin America indicating the countries where freshwater invertebrate and vertebrate hosts, of four classes, have been studied for neotropic symbiotic temnocephalans. Countries with major to 10 species records in dark grey and minor to 2 in low grey. The numbers in circles indicate the total temnocephalan taxa recorded. Map produced by http://www.naturalearthdata.com/, and modified in DIVA-GIS 7.5 (Hijmans et al. 2012) (freely available through www.diva-gis.org).
This checklist presents data on almost all the extant species of temnocephalans along their distributional ranges in 11 Neotropical countries, which represents 35% of the total political territories (i.e. countries) in the Neotropics (Figure 2). Argentina, Brazil and Uruguay are the countries with the most records of temnocephalans and with the most endemic species of _Temnocephala_, which are represented by 6, 9 and 4 species, respectively, while Colombia, Costa Rica, Mexico and Peru hold 1, 2, 1 and 1 endemic species, respectively. The relatively high number of records in Argentina, Brazil and Uruguay can imply that in these countries there are more research groups working with turbellarians compared to other Neotropical countries (e.g. Damborenea and Brusa 2008, Volonterio 2010, Amato et al. 2011). Therefore, the values of endemism for these particular countries are subjective – a function of the research effort – and it is probable that the endemism may be increased/decreased in future studies from different Neotropical countries. With regards to its exclusively Neotropical distribution, morphological evidence (mosaic syncytial plates) (e.g. Cannon and Joffe 2001, Damborena and Cannon 2001b), plus the recorded host specificities shown in this study (Appendix 1), allow for the inference that the biological radiation of _Temnocephala_ may be the result of a complex combination of ancestral allopatric speciation processes (as a result of the separation of South America and Australia), plus the diversification of their host groups (e.g. Parastacidae) in South and subsequent radiation in Central America. For example, the species of _Temnocephala_ associated with mollusks appear to be a morphologically homogeneous group with a phylogenetic structure (Volonterio 2007a, Damborena and Brusa 2008). On the other hand, the almost exclusive distribution in the Southern Hemisphere of the family to which _Temnocephala_ belongs (Temnocephalidae) is noteworthy and alludes to a Gondwanian origin (Gelder 1999, Cannon and Joffe 2001). However, a reliable molecular clock of the Temnocephalida is required to support or reject this hypothesis. Future studies combining research programs in integrative taxonomy (Schlick-Steiner et al. 2010, Ceccarelli et al. 2012, Fujita et al. 2012) with approaches of historical association (e.g. genes, organism and areas; see Page and Charleston 1998) will decipher the evolutionary history of _Temnocephala_.

At least 60 papers have been published dealing with the records of Neotropical symbiotic temnocephalans; however, the scarcity of studies in many countries is clear, and needs to be rectified. For example, some countries comprising complex geographic areas (i.e. Mexican Transition Zone, South American Transition Zone) only have one record of these turbellarians, and the diversity of the four major host groups is also unknown (Martínez-Aquino et al. 2014a). Therefore, we contend that future survey work should be strategic, aimed at enhancing the biodiversity inventory, combining identification of the host spectrum with choice of appropriate drainages based on biogeographic, faunistic, and hydrologic data and on lessons from other freshwater symbiotic Platyhelminthes (e.g. Pérez-Ponce de León and Choudhury 2010, Martínez-Aquino et al. 2014c).

Acknowledgements

We thank F.S. Ceccarelli, R. Pérez-Rodriguez, L. Alcalde and L. Negrete for their help during field work. L. García-Prieto for providing literature and specimens for morphological comparison deposited at the CNHE. J. Vigliano Relva help to obtained geographical coordinates. We thank Sean M. Rovito for help with language editing and two reviewers for their constructive criticisms. A.M-A. is currently supported by a postdoctoral grant from CONACYT, Mexico (No. 207983). This study was supported by grants PIP CONICET 0390 and FCNyM-UNLP N11/728 to C.D. and PIP CONICET 11220120100635CO F.B.

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Supplementary material

Appendix 1. Host-symbiont temnocephalans list from Neotropics
Authors: Andrés Martínez-Aquino, Francisco Brusa, Cristina Damborenea
Data type: Microsoft Excel file (xls)
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Common littoral pycnogonids of the Mediterranean Sea

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Abstract

In the present study 21 littoral pycnogonid species from 5 families are analysed: Ammothelidae (9 species), Callipallenidae (5 species), Endeidae (2 species), Phoxichilidiidae (3 species), and Pycnogonidae (2 species). The material was mainly taken from Mediterranean pycnogonid collections housed in the Bavarian State Collection of Zoology. Additional material was collected during field trips. Altogether the material was obtained from six different locations: Banyuls-sur-Mer (France), Tavolara Island (Italy), Elba Island (Italy), Giglio Island (Italy), Sicily Island (Italy), and Istria Peninsula (Croatia). The animals were studied in detail with a scanning electron microscope (SEM). Additionally series of light microscopic pictures were made to generate extended depth of field pictures of whole animals. The observed features are compared with previous literature.

Key Words

SEM
Pycnogonida
Sea spider
\textit{Achelia}
\textit{Ammothella}
\textit{Anoplodactylus}
\textit{Callipallene}
\textit{Endeis}
Pycnogonum
Tanystylum
Trygaeus

Introduction

The Pycnogonida or sea spiders are exclusively marine invertebrates, numbering more than 1330 species worldwide (Arango and Wheeler 2007, Bamber and El Nagar 2013). They were first named \textit{Pycnonides} Latreille, 1810. Later they were given the names \textit{Podosomata} Leach, 1815 and \textit{Pantopoda} Gerstaecker, 1863. The latter name is still in use for the order which comprises all extant pycnogonids. Although largely unnoticed, due to their cryptic form, often small size, and economic insignificance, sea spiders are common benthic animals occurring from the littoral zone to the deep-sea, in all oceans from the poles to the tropics. Most species are benthic, few are interstitial, some are pelagic and some are commensals or ectoparasites of other invertebrates. Pycnogonids are normally small animals; littoral species have a leg span of at most a few centimetres, while polar and deep-water species can achieve a leg span of 70 cm (Ruppert et al. 2004).

The phylogenetic position of the Pycnogonida has long been controversial and is still under debate. Today pycnogonids are placed either within the Chelicerata as sister taxon of the Euchelicerata or as sister taxon of all other Euarthropoda (Dunlop and Arango 2005, Regier et al. 2010, Giribet and Edgecombe 2012). The phylogenetic relationships within the group are discussed as well. Traditionally the Pycnogonida were divided into eight families (Hedgpeth 1947) but with uncertain relationships.
Recently studies on this point, using morphological and/or molecular characters were realized (Arango 2002, Arango 2003, Arango and Wheeler 2007, Nakamura et al. 2007). On the basis of these studies Bamber (2007) and Bamber and El Nagar (2013) suggested 11 families.

The Mediterranean Sea as we know it today developed about 5 million years ago. After the Messinian salinity crisis (5.6 to 5.3 Mya) the Mediterranean Sea was filled in a major flood approximately 5.3 million years ago, in which water poured in from the Atlantic Ocean and through the Strait of Gibraltar (Tichy et al. 2001). Consequently, the Mediterranean marine biota including the pycnogonid fauna is derived primarily from the Atlantic Ocean. The opening of the Suez Canal in 1869 created the first salt-water passage between the Mediterranean and Red Sea allowing some Red Sea species to invade the Mediterranean (lessepsian migration) (Schmidt et al. 2001). *Anoplodactylus californicus, A. digitatus*, and *Pi- grogromitus timsanus* are regarded as being lessepsian migrants (Chimenz-Gusso and Lattanzi 2003).

In the Mediterranean Sea 56 pycnogonid species are known until now. A current species list of the Mediterranean pycnogonid fauna is found in Chimenz-Gusso and Lattanzi (2003), with the completion of *Anoplodactylus namus*, described by Krapp, Kocak, and Katagan in 2008 (Krapp et al. 2008). The Mediterranean pycnogonid fauna consists mostly of littoral species, and deep-sea species are an exception. It is assumed that, because of the geological barrier caused by the Strait of Gibraltar (max. depth 286 m) and the temperature barrier in the deep sea (Atlantic 1–4°C, Mediterranean 12°C), the deep water fauna in the Mediterranean is relatively species-poor (Schmidt et al. 2001). Deep-sea pycnogonid species adapted to cold water such as representatives of the Colossendeidae and Pallenopsidae are either absent or rarely occur in the Mediterranean. A similar barrier also exists between the western and eastern basin of the Mediterranean and between the Mediterranean Sea and the Black Sea.

Mediterranean pycnogonid literature is scattered (e.g. Giltay 1929, Helfer 1936, Faraggiana 1940, Bourdillon 1954, Stock 1958, Bacsciu 1959, Stock 1962, Stock and Soyer 1965, Stock 1966, Zavodnik 1968, Krapp 1969, Schüller 1989, Pérez-Ruzafa and Munilla 1992, Chimenz et al. 1993, Munilla 1993, Munilla and Nieto 1999, Chimenz-Gusso 2000, Montoya Bravo et al. 2006, Krapp et al. 2008, Kocak et al. 2010, Kocak and Alan 2013). Of particular significance is Dohrn’s (1881) “Die Pantopoden des Golfes von Neapel und der angrenzenden Meeresab- schnitte” with its excellent drawings and descriptions of 18 new species. The first detailed comprehensive taxonomic work about sea spiders in the Mediterranean was by Bouvier (1923), which also dealt with Atlantic species. Zoogeographical studies were done by Arnaud (1987) and Chimenz-Gusso and Lattanzi (2003); the deep-water species were discussed by Stock (1987). As many of the species distributed in Mediterranean are of Atlantic origin, also the survey of Bamber (2010) is of special interest.

There is a rapidly growing body of molecular studies especially on the Antarctic and Subantarctic fauna (e.g. Mahon et al. 2008, Nielsen et al. 2009, Krabbe et al. 2010, Dietz et al. 2011, Arango et al. 2011, Weis and Melzer 2012b, Dietz et al. 2013, Carapelli et al. 2013). In contrast, there is a lack of publications with molecular studies for the Mediterranean. However, so far – as at January 2014 – 11 Mediterranean pycnogonid species are listed in BOLD (Ratnasingham and Hebert 2007) and 10 species in GenBank (Benson et al. 2010). Detailed morphological analyses of Mediterranean Sea species are required to support future molecular studies, and specimens stored in natural history collections can provide a useful basis for these studies (see also Dunlop et al. 2007, Weis et al. 2011, Weis and Melzer 2012a).

In the present study representatives of some of the major genera of Mediterranean pycnogonids were sourced from the Bavarian State Collection of Zoology. Additional material was collected during field trips. A significant objective of this study is to remove any ambiguity in species identifications by providing a pictorial atlas principally based on high resolution Scanning Electron Microscope (SEM) images. Classification follows Bamber (2007) and PycnoBase (Bamber and El Nagar 2013).

**Material and methods**

**Material:** The majority of specimens were sourced from the Bavarian State Collection of Zoology. Additional material was collected during field trips to Banyuls-sur-Mer, France (June/July 2006) and Rovinj, Croatia (September 2006 and May 2007). Collecting sites are summarized in Figure 1. Details are given under material section of each species. All material was conserved and stored in 75% ethanol. Preparations were made according to methods described in Bolte (1996). Only adult animals were used for light microscopic and SEM imaging. Species determinations are based on the original descriptions and a variety of literature suitable for Mediterranean pycnogonids (e.g. Dohrn 1881, Bouvier 1923, Stock 1968, Bamber 2010). Synonyms followed PycnoBase (Bamber and El Nagar 2013) and Müller (1993). All specimens (SEM and alcohol material) used for this study are deposited at the Bavarian State Collection of Zoology.

**Light microscopy:** Light microscopic pictures were taken using an Olympus SZX stereo microscope and a Jenopttc Prog-Res C12 digital camera (2580 × 1944 px; 96 dpi; colour depth 24 bit). Larger specimens (*Ende- is charybdaea, E. spinosa*) were photographed using a Wild-Heerbrugg M5A stereo microscope and a Canon Digital IXUS 850 IS digital camera (3072 × 2304 px; 180 dpi; colour depth 24 bit). Up to 12 pictures with different focus steps along the z-axis were combined to a single respective image with a greater field of depth using the computer software Auto Montage (Syncroscopy) or Helicon Focus (HeliconSoft).

**Scanning electron microscopy:** For SEM preparation, specimens were dehydrated in a graded acetone series (70%, 80%, 90%, 10 min. each, plus 3 × 100%, 20 min.
each) and critical-point-dried in a Baltec CPD 030. Dried specimens were mounted on SEM stubs with self-adhesive carbon stickers and coated with gold on a Polaron Sputter Coater. SEM pictures (2048 × 1536 px; 72 dpi; colour depth 8 bit) were made with a LEO 1430VP at 10–20 kV. Scales were inserted using the measurement utility of the SEM.

**Nomenclature:** The present study follows the nomenclature in Arnaud and Bamber (1987) and Bamber (2010). Hence, the trunk of a pycnogonid is divided into 4 segments, the cephalon (=segment 1) and the segments 2, 3, and 4. The cephalon carries the proboscis anteroventrally, the ocular tubercle dorsally, and four pairs of extremities: the chelifores above the proboscis, the palps laterally, the ovigers ventrally, and one pair of legs laterally. Segments 2, 3, and 4 each carry one pair of legs laterally. The fourth segment carries the abdomen posterodorsally. Some species do not possess palps or chelifores. Ovigers are present in males (with few exceptions, e.g. *Pycnogonum* subgen. *Nulloviger*). In some genera ovigers are reduced or absent in females. The number of articles of the chelifores, palps, and ovigers varies within the systematic groups. Each leg is composed of nine articles: first, second, and third coxa, femur, first and second tibiae, tarsus, propodus, and claw. The first coxa articulates with the lateral process of the trunk while the second coxa carries the genital opening ventrally. In males, the cement glands are on the femur and rarely on other articles. The claw is often flanked by two smaller auxiliary claws. All abbreviations used in the figures are provided in Table 1.

**Results**

**General remarks**

This study is based on 21 Mediterranean species. In all cases, the major morphological characteristics correspond with published descriptions; exceptions see remarks section of each species.

**Classification**

Order Pantopoda Gerstäcker, 1863
Suborder Eupantopodida Fry, 1978
Superfamily Ascorhynchoidea Pocock, 1904
Family Ammotheidae Dohrn, 1881
*Achelia echinata* Hodge, 1864
*Achelia langi* (Dohrn, 1881)
*Achelia vulgaris* (Costa, 1861)
*Ammothella appendiculata* (Dohrn, 1881)
*Ammothella biunguiculata* (Dohrn, 1881)
*Ammothella longipes* (Hodge, 1864)
*Ammothella uniuunguiculata* (Dohrn, 1881)
*Tanystylum conirostre* (Dohrn, 1881)
*Trygaeus communis* Dohrn, 1881
Superfamily Nymphonoidea Pocock, 1904
Family Callipallenidae Hilton, 1942
*Callipallene emaciatia* (Dohrn, 1881)
*Callipallene phantoma* (Dohrn, 1881)

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**Callipallene producta** (Sars, 1888)
**Callipallene spectrum** (Dohrn, 1881)
**Callipallene tiberi** (Dohrn, 1881)

Superfamily Phoxichilioidea Sars, 1891
Family Endeiidae Norman, 1908
*Endeis charybdae* (Dohrn, 1881)
*Endeis spinosa* (Montagu, 1808)

Family Phoxichiliidiidae Sars, 1891
*Anoplodactylus angulatus* (Dohrn, 1881)
*Anoplodactylus petiolatus* (Krøyer, 1844)
*Anoplodactylus pygmaeus* (Hodge, 1864)

Superfamily Pycnogonoidea Pocock, 1904
Family Pycnogonidae Wilson, 1878
*Pycnogonum nodulosum* Dohrn, 1881
*Pycnogonum (Retroviger) pusillum* Dohrn, 1881

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**Ammotheidae Dohrn, 1881**

*Achelia Hodge, 1864*

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*Achelia echinata* Hodge, 1864

Figures 2, 5–8

**Synonyms:** *Ammothea brevipes* Hodge, 1864
*Ammothea echinata* Hodge, 1864
*Ammothea fibulifera* (Dohrn, 1881)

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**Material.** ZSMA20071461: male; Giglio Island, Italy; 08.04.2005; 4–8 m; brown algae. ZSMA20071462: female; Giglio Island, Italy; 11.04.2005; 20 m. ZSMA20071463: female; Rovinj, Croatia; 05.07.2005; 0–1.5 m; *Stypocaulon scoparium* (Linnaeus) Kützing, 1843.

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*Achelia Langi* (Dohrn, 1881)

Figures 2, 9, 10

**Synonyms:** *Ammothea langi* Dohrn, 1881

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**Material.** ZSMA20071464: male; Cape Savudrija, Croatia; 08.10.2004: 0–1 m; under stones. ZSMA20071465: male; Cape Savudrija, Croatia; 22.05.2005: 0–1 m. ZSMA20071466: female; Cape Savudrija, Croatia; 08.08.2005; 0–1 m; on stone.

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**Remarks.** According to Dohrn (1881) the lateral processes 1–3 are armed with one protuberance with spine. Here, in the male, the lateral process 1 has two protuberances with spine and the lateral process 2 and 3 have one (Fig. 9D, E). The lateral processes in the female correspond with Dohrn’s description.

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*Achelia vulgaris* (Costa, 1861)

Figures 2, 11, 12

**Synonyms:** *Achelia franciscana* (Dohrn, 1881)
*Alcinous vulgaris* Costa, 1861
*Ammothea franciscana* Dohrn, 1881

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**Material.** ZSMA20071467: male; Bayuls-sur-Mer, France; 07.2004. ZSMA20071468: female; Giglio Island, Italy;
08.04.2005; 8–13 m; brown algae. ZSMA20071469: female; Giglio Island, Italy; 08.04.2005; 8–13 m; brown algae.

**Remarks.** Adult males are differentiated by the character described by Dohrn (1881) and Bouvier (1923): three protuberances with spine on each side of coxa 2. But Dohrn notes that there also exist varieties: two protuberances on each side of coxa 2 or two protuberances on one and three protuberances on the other side of coxa 2.

Here, only coxa 2 of the first leg has three protuberances with spine on the one and two on the other side; the coxa 2 of the other legs has two protuberances with spine on each side (Fig. 11C, D). The other characters of the male, like lateral process not touching each other (Fig. 11B) correspond with the descriptions of Costa (1861), Dohrn (1881) and Bouvier (1923).

**Ammothella** Verrill, 1900

**Ammothella appendiculata** (Dohrn, 1881)

Figures 3, 13, 14

**Synonyms:** Ammoothea appendiculata Dohrn, 1881

**Material.** ZSMA20071470: female; Cape Savudrija, Croatia; 03.09.2004: 0–1 m. ZSMA20071471: female; Cape Savudrija, Croatia; 08.10.2004: 0–1 m; on stone. ZSMA20071472: female; Cape Savudrija, Croatia; 08.08.2005; 0–1 m; under stone.

**Remarks.** Dohrn (1881) described the palp with 10 articles; here the palp, as in other Ammothella, has 9 articles (Fig. 13E).

**Ammothella biunguiculata** (Dohrn, 1881)

Figures 3, 15, 16

**Synonyms:** Ammoothea biunguiculata Dohrn, 1881

**Material.** ZSMA20071473: male; Cape Savudrija, Croatia; 08.10.2004: 0–1 m; on stone.

**Ammothella longipes** (Hodge, 1864)

Figures 3, 17

**Synonyms:** Achelia longipes (Hodge, 1864)

Achelia magnirostris (Dohrn, 1881)

Ammothella longipes Hodge, 1864

Ammothella magnirostris (Dohrn, 1881)

**Material.** ZSMA20071477: female; Bayuls-sur-Mer, France; 07.2004.

**Ammothella uniunguiculata** (Dohrn, 1881)

Figures 4, 18

**Synonyms:** Ammoothea uniunguiculata Dohrn, 1881

**Material.** ZSMA20140003: male; Sicily, Italy; 1988

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**Tanystylum Miers, 1879**

**Tanystylum conirostre** (Dohrn, 1881)

Figures 4, 19–21

**Synonyms:** Clotenia conirostris Dohrn, 1881

**Material.** ZSMA20071505: female; Bayuls-sur-Mer, France; 07.2004. ZSMA20071506: female; Bayuls-sur-Mer, France; 07.2004. ZSMA20071507: male; Bayuls-sur-Mer, France; 07.2004. ZSMA20071508: male; Bayuls-sur-Mer, France; 07.2004.

**Trygaeus Dohrn, 1881**

**Trygaeus communis** Dohrn, 1881

Figures 4, 22–25

**Material.** ZSMA20071509: female; Giglio Island, Italy; 12.04.2005. ZSMA20071510: male; Giglio Island, Italy; 12.04.2005. ZSMA20071511: male; Giglio Island, Italy; 12.04.2005. ZSMA20071512: male; Giglio Island, Italy; 12.04.2005. ZSMA20071513: male; Cape Kamenjak, Croatia; 03.05.2007; 0–5 m; *Stypocaulon scoparium* (Linnaeus) Küting, 1843.

**Remarks.** According to Dohrn (1881), Bouvier (1923) and Stock (1966) the palps vary in the number of articles from 4 to 7 and the oviger from 6 to 10. Here the palp has 7 articles (Fig. 23A) and the male and female oviger have 9 articles (Fig. 23C, D; Fig. 25D). Other specimens not depicted have four palp articles.

**Callipallenidae Hilton, 1942**

**Callipallene Flynn, 1929**

**Callipallene emaciata** (Dohrn, 1881)

Figures 26, 28, 29

**Synonyms:** Callipallene emaciata emaciata Stock, 1954

Callipallene emaciata (Dohrn, 1881)

**Pallene emaciata** Dohrn, 1881

**Material.** ZSMA20071485: female; Giglio Island, Italy; 09.04.2005; 3–5 m; *Halopteris* sp.. ZSMA20071486: female; Giglio Island, Italy; 09.04.2005; 3–5 m; *Halopteris* sp.. ZSMA20071487: male; Giglio Island, Italy; 09.04.2005; 3–5 m; *Halopteris* sp..

**Callipallene phantoma** (Dohrn, 1881)

Figures 26, 30–33

**Synonyms:** Callipallene phantoma crinita Stock, 1952

Callipallene phantoma phantoma (Dohrn, 1881)

**Pallene phantoma** Dohrn, 1881

**Material.** ZSMA20071489: female; Brucoli, Sicily, Italy; 14.09.1988. ZSMA20071490: female; Bayuls-sur-mer, France; 07.2004. ZSMA20071491: female; Giglio Island, Italy; 11.04.2005; 20 m; red algae. ZSMA20071492: male; Giglio Island, Italy; 11.04.2005; 20 m; red algae.
**ZSMA20071493:** male; Giglio Island, Italy; 11.04.2005; 20 m; red algae. ZSMA20071494: male; Rovinj, Croatia; 05.07.2005.

**Remarks.** Dohrn (1881) describes the auxiliary claws as half as long as the claw. According to Stock (1952) the length of auxiliary claws can vary between specimens. Here all 6 specimens from 4 different locations have more or less rudimentary auxiliary claws (Fig. 31F; Fig. 33F, G).

**Callipallene producta** (Sars, 1888)

Figures 26, 34, 35

**Synonyms:**
- Callipallene brevirostris producta (Sars, 1888)
- Pallene producta Sars, 1888

**Material.**
- ZSMA20071495: female; Giglio Island, Italy; 08.04.2005; 13–18 m; on brown algae. ZSMA20071496: female; Giglio Island, Italy; 08.04.2005; 13–18 m; on brown algae.

**Callipallene spectrum** (Dohrn, 1881)

Figures 27, 36

**Synonyms:**
- Callipallene emaciata spectrum (Dohrn, 1881)
- Pallene spectrum Dohrn, 1881

**Material.**
- ZSMA20140004: male; Banjole, Rovinj, Croatia; 07.1968; 5 m; on *Cystoseira* sp.

**Callipallene tiberi** (Dohrn, 1881)

Figures 27, 37

**Synonyms:**
- Callipallene emaciata tiberii (Dohrn, 1881)
- Pallene tiberi Dohrn, 1881

**Material.**
- ZSMA20071488: female; Giglio Island, Italy; 12.04.2005; 1 m; *Halopteris* sp.

**Endeidae Norman, 1908**

**Endeis Philippi, 1843**

**Endeis charybdaeae** (Dohrn, 1881)

Figures 38–40

**Synonyms:**
- Chilophoxus charybdaeae (Dohrn, 1881)
- Phoxichilus charybdaeae Dohrn, 1881

**Material.**
- ZSMA20071497: male; Rovinj, Croatia; 04.2004. ZSMA20071498: female; Rovinj, Croatia; 04.2004. ZSMA20071499: male; Rovinj, Croatia; 05.05.2007; 0–2 m; *Corallina elongata* J.Ellis & Solander, 1786.

**Remarks.** According to Dohrn (1881) and Bamber (2010) each lateral process and coxa 1 is armed with one protuberance. Here, in the males the lateral process 1 and the coxa 1 of the first leg has two protuberances (Fig. 42D, F), the remaining legs correspond with previous observations (e.g. Fig. 42E, G). The females are without such protuberances (Fig. 44C, D).

**Phoxichilidiidae Sars, 1891**

**Anoplodactylus Wilson, 1878**

**Anoplodactylus angulatus** (Dohrn, 1881)

Figures 45–47

**Synonyms:**
- Anoplodactylus angulirostris (Dohrn, 1881)
- Phoxichilidium angulatum Dohrn, 1881

**Material.**
- ZSMA20071478: female; Banyuls-sur-mer, France; 07.2004. ZSMA20071479: female; Giglio Island, Italy; 14.4.2005; 20 m; red algae.

**Anoplodactylus petiolatus** (Krøyer, 1844)

Figures 45, 48

**Synonyms:**
- Anoplodactylus derjugini (Losina-Losinsky, 1929)
- Anoplodactylus exiguus (Dohrn, 1881)
- Pallene pygmaea Hoek, 1881
- Pallene attenuata Hodge, 1864
- Phoxichilidium exiguum Dohrn, 1881
- Phoxichilidium pygmaeum Hoek, 1881
- Phoxichilidium petiolatum Krøyer, 1844

**Material.**
- ZSMA20071480: male; Banyuls-sur-mer, France; 07.2004. ZSMA20042384: male; Bayuls-sur-Mer, France; 06.2003.

**Remarks.** In literature there is diverse information about the articulation of the oviger, 7 articles in Dohrn (1881) and 6 articles in Bouvier (1923) and King (1986) are reported. Here the ovigers have 6 articles.

**Anoplodactylus pygmaeus** (Hodge, 1864)

Figures 45, 49–52

**Synonyms:**
- Anoplodactylus derjugini (Losina-Losinsky, 1929)
- Anoplodactylus exigus (Dohrn, 1881)
- Pallene pygmaeae Hodge, 1864
- Phoxichilidium exiguum Dohrn, 1881
- Phoxichilidium pygmaeum Hoek, 1881

**Material.**
- ZSMA20071481: female; Elba, Italy; 20.10.2004; 3–5 m. ZSMA20071482: female; Elba, Italy; 21.10.2004; 3–5 m. ZSMA20071483: male; Elba, Italy; 20.10.2004; 0.5–4 m. ZSMA20071484: male; Cape
Kamenjak, Croatia; 03.05.2007; 0–5 m; *Stypocaulon scoparium* (Linnaeus) Kützing, 1843.

**Remarks.** In the literature there is diverse information about the articulation of the oviger, 7 articles in Dohrn (1881) and 6 articles in Bouvier (1923) and King (1986) are reported. Here the ovigers have 6 articles (Fig. 50E, F).

**Pycnogonidae Wilson, 1878**
**Pycnogonum Bruennich, 1764**
**Pycnogonum nodulosum** Dohrn, 1881
Figures 53, 54

**Material.** ZSMA20071503: female; Banyuls-sur-mer, France; 07.2004; 30 m; red algae. ZSMA20030112: female; Tavolara Island, Sardinia, Italy; 2002.

**Pycnogonum** *(Retroviger)* *pusillum* Dohrn, 1881
Figures 53, 55
Synonyms: *Pycnogonum pusillum* Dohrn, 1881

**Material.** ZSMA20071504: male; Brucoli, Sicily, Italy; 14.09.1988.

**Figures**

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**Figure 1.** Collecting sites of pycnogonids used in this study: 1: Banyuls-sur-Mer (France); 2: Tavolara Island (Italy); 3: Elba Island (Italy); 4: Giglio Island (Italy); 5: Brucoli, Sicily (Italy); 6: Istria Peninsula (Croatia).
Figure 2. Amмоtheidae 1; A, B: Achelia echinata, male, dorsal view; scales 500 µm and 250 µm, respectively; C, D: Achelia langi, male, dorsal view; scales 1 mm and 500 µm, respectively; E, F: Achelia vulgaris, male, dorsal view; scales 1 mm and 250 µm, respectively.
Figure 3. Ammotheidae 2; **A, B**: *Ammothella appendiculata*, female, dorsal view; scales 1 mm and 250 µm, respectively; **C, D**: *Ammothella biunguiculata*, male, dorsal view; scales 1 mm and 250 µm, respectively; **E, F**: *Ammothella longipes*, female, dorsal view; scales 1 mm and 250 µm, respectively.
Figure 4. Ammotheidae 3; A, B: *Ammothella uniunguiculata*, male, dorsal view; scales 200 µm; C, D: *Tanystylum conirostre*, male, dorsal view; scales 1 mm and 250 µm, respectively; E, F: *Trygaeus communis*, male, dorsal view; scales 1 mm and 250 µm, respectively.
**Figure 5. Achelia echinata***, male; **A**: Dorsal view; scale 400 µm; **B**: Dorsal view of trunk, lateral processes touch each other; scale 200 µm; **C**: Frontal view of trunk, protuberances with spines on lateral process; scale 200 µm; **D**: Lateral view of proboscis, palp and chelifores with reduced chela; scale 100 µm; **E**: Mouth opening, dorsal is up; scale 20 µm; **F**: Mouth opening; scale 5 µm.
Figure 6. *Achelia echinata*, male; **A**: Chelifores with reduced chela; scale 40 µm; **B**: Distal articles of right 8-articled palp; scale 40 µm; **C**: Distal articles of 10-articled oviger; scale 40 µm; **D, E**: Compound spines on last oviger-article; scales 10 µm and 5 µm, respectively; **F**: Lateral process, coxa 1 and 2 of right 3rd leg, 2 protuberances with spine on each side of coxa 2; scale 100 µm; **G**: Coxa 2 with 2 protuberances with spine on each side (right 3rd leg); scale 40 µm.
Figure 7. *Achelia echinata*, male; A: Left 3rd leg; scale 200 µm; B: Lateral view of coxa 2 with genital protuberance (right 4th leg); scale 40 µm; C: Genital opening (right 3rd leg); scale 20 µm; D: Lateral view of femur with cement gland on distal part (left 3rd leg); scale 100 µm; E: Cement gland (right 4th leg); scale 40 µm; F: Tarsus, propodus, and claw, auxiliary claws about half as long as claw (left 3rd leg); scale 100 µm; G: Abdomen; scale 40 µm; H: Hair and slit organ on dorsal side of trunk; scale 5 µm.
Figure 8. *Achelia echinata*, female; A: Dorsal view; scale 400 µm; B: Ventral view of proboscis; scale 100 µm; C: Left 10-articled oviger; scale 100 µm; D: Lateral process, coxa 1 and 2 of right 3rd leg, lateral processes touch each other; scale 100 µm; E: Left 3rd leg; scale 200 µm; F: Ventral view of coxa 2 with genital opening, distal is right (left 3rd leg); scale 40 µm; G: Genital opening; scale 20 µm.
Figure 9. *Achelia langi*, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 400 µm; C: Ventral view of proboscis; scale 200 µm; D: Dorsal view of right lateral processes 1 and 2, 2 protuberances with spine on lateral process 1 and 1 on lateral process 2; scale 100 µm; E: Dorsal view of right lateral processes 3 and 4, 1 protuberance with spine on lateral process 3 and lateral process 4 without protuberance or spine; scale 100 µm; F: Left 3rd leg; scale 400 µm; insert: Genital protuberance; scale 100 µm.
Figure 10. *Achelia langi*, female; A: Dorsal view of trunk; scale 400 µm; B: Dorsal view of left lateral processes, 1 protuberance with spine on lateral process 1-3, lateral process 4 without protuberance or spine; scale 200 µm; C: Right 3rd leg; scale 400 µm; D: Ventral view of coxa 2 with genital opening, distal is down (right 3rd leg); scale 100 µm; E: Genital opening; scale 20 µm; F: Tarsus, propodus, and claw, auxiliary claws about 2/3 of length of claw; scale 100 µm; G: Abdomen with anus; scale 40 µm.
Figure 11. *Achelia vulgaris*, male; A: Dorsal view of trunk; scale 400 µm; B: Dorsal view of left lateral processes, lateral processes do not touch each other; scale 200 µm; C: 2 protuberances with spine on right side and 3 on left side of coxa 2, distal is down (right 1st leg); scale 100 µm; D: Lateral process, coxa 1 and 2, 2 protuberances with spine on each side of coxa 2 (left 3rd leg); scale 100 µm; E: Right 3rd leg; scale 400 µm; F: Tarsus, propodus, and claw, auxiliary claws about half as long as claw; scale 100 µm.
Figure 12. *Achelia vulgaris*, female; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk, lateral processes do not touch each other; scale 300 µm; C: Ventral view of proboscis; scale 100 µm; D: Right 10-articled oviger; scale 100 µm; E: Right 3rd leg; scale 400 µm; F: Dorsal view of coxa 2 (left 3rd leg); scale 40 µm; G: Ventral view of coxa 2 with genital opening (left 3rd leg); scale 40 µm.
Figure 13. *Ammothella appendiculata*, female; A: Dorsal view of trunk; scale 400 µm; B: Lateral view of trunk; scale 200 µm; C: Ventral view of trunk; scale 200 µm; D: Mouth opening, dorsal is up; scale 20 µm; E: Right chelifere with reduced chela and right 9-articled palp; scale 100 µm; F: Ocular tubercle with lateral sense organ; scale 40 µm.
Figure 14. *Ammothella appendiculata*, female; A: Lateral process of left 3rd leg; scale 40 µm; B: Coxa 1 of right 3rd leg with hollow spines; scale 40 µm; C: Hollow spine; scale 5 µm; D: Right 4th leg; scale 400 µm; E: Tarsus, propodus, and claws (right 4th leg); scale 100 µm; F: Hairs and slit organs of dorsal side of trunk; scale 10 µm.
Figure 15. Ammothella biunguiculata, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 400 µm; C: Lateral view of trunk, oviger dissected; scale 400 µm; D: Dorsal view of proboscis; scale 100 µm; E: Right chelifore with reduced chela; scale 40 µm; F: Right 9-articled palp; scale 100 µm.
Figure 16. *Ammothella biunguiculata*, male; A: Lateral view of ocular tubercle; scale 100 µm; B: Right 3rd leg; scale 400 µm; C: Ventral view of coxa 2 with genital opening, distal is left (right 3rd leg); scale 40 µm; D: Tarsus and propodus with reduced claw and dominating auxiliary claws; scale 100 µm; E: Reduced claw and dominating auxiliary claws; scale 20 µm; F: Dorsal view of trunk segment 4 and abdomen; scale 100 µm.
Figure 17. *Ammothella longipes*, female; A: Dorsal view of trunk; scale 200 µm; B: Lateral view of trunk, one protuberance without spine aside on lateral processes; scale 200 µm; C: Ocular tubercle; scale 100 µm; D: Right lateral process; scale 100 µm; E: Left 3rd leg; scale 400 µm; F: Tarsus, propodus, and claws (left 3rd leg); scale 100 µm.
Figure 18. *Ammotheilla unisuniculata*, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 100 µm; C: Cephalon with proboscis, chelifore, and palps; scale 200 µm; D: Right 3rd leg; scale 200 µm; E: Tibia 2, tarsus, and propodus with claw (right 3rd leg); scale 100 µm; F: Propodus with claw; scale 20 µm.
Figure 19. Tanystylum conirostre, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 200 µm; C: Dorsal view of proboscis and 4-articled palp; scale 100 µm; D: Mouth opening; scale 20 µm; E: Reduced chelifores, frontal is up; scale 40 µm; F: Right 10-articled oviger; scale 100 µm.
Figure 20. Tanystylum conirostre, male; A: Distal articles of right oviger with spines on tip; scale 10 µm; B: Left 3rd leg; scale 100 µm; C: Lateral view of coxa 2 with genital opening on a protuberance, distal is right (left 4th leg); scale 40 µm; D: Genital opening; scale 10 µm; E: Cement gland on femur, distal is up (left 3rd leg); scale 20 µm; F: Tarsus, propodus, and claws (left 3rd leg); scale 100 µm; G: Hairs and slit organs on dorsal side of trunk; scale 10 µm.
Figure 21. *Tanystylum conirostre*, female; A: Dorsal view of trunk; scale 400 µm; B: Frontal view of trunk; scale 200 µm; C: Left 10-articled oviger; scale 40 µm; D: Distal articles of left oviger; scale 40 µm; E: Ventral view of coxa 2 with genital opening, distal is right (right 4th leg); scale 40 µm; F: Genital opening; scale 10 µm; G: Abdomen; scale 40 µm.
Figure 22. *Trygaeus communis*, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 400 µm; C: Lateral view of trunk with protuberances on midline; scale 400 µm; D: Mouth opening; scale 40 µm; E: Dorsal view of proboscis and chelifore without chela; scale 100 µm; F: Tip of chelifore without chela; scale 20 µm.
Figure 23. *Trygaeus communis*, male; A: Lateral view of proboscis and 7-articled right palp; scale 100 µm; B: Ocular tubercle with lateral sense organ; scale 40 µm; C: Right 9-articled oviger; scale 100 µm; D: Distal articles of right oviger; scale 40 µm; E: Protuberance on midline of segment 2; scale 40 µm; F: Right 3rd leg; scale 200 µm.
Figure 24. *Trygaeus communis*, male; A: Dorsal view of coxa 1 with protuberances on distal corners (right 3rd leg); scale 100 µm; B: Ventral view of coxa 2 with genital opening, distal is down (left 3rd leg); scale 40 µm; C: Genital opening; scale 10 µm; D: Cement gland on femur, distal is left (left 3rd leg); scale 40 µm; E: Tarsus, propodus, and claws (right 3rd leg); scale 100 µm; F: Abdomen with protuberance on tip; scale 100 µm; G: Hairs and slit organs on dorsal side of trunk; scale 10 µm.
Figure 25. *Trygaeus communis*, female; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 300 µm; C: Lateral view of trunk with protuberances on midline, frontal is right; scale 100 µm; insert: Protuberance on segment 2; scale 20 µm; D: Distal article of oviger; scale 40 µm; E: Dorsal view of coxa 1 without protuberances on distal corners (right 2nd leg); scale 40 µm; F: Ventral view of coxa 2 with genital opening, distal is down (right 3rd leg); scale 100 µm; G: Genital opening; scale 20 µm.
Figure 26. Callipallenidae 1; A, B: Callipallene emaciata, female, dorsal view; scales 1 mm and 250 µm, respectively; C, D: Callipallene phantoma, male, dorsal view; scales 1 mm and 250 µm, respectively; E, F: Callipallene producta, female, dorsal view; scales 1 mm and 250 µm, respectively.
Figure 27. Callipallenidae 2; A, B: *Callipallene spectrum*, male, dorsal view; scales 200 μm; C, D: *Callipallene tiberi*, female, dorsal view; scales 500 μm and 250 μm, respectively.
Figure 28. *Callipallene emaciata*, male; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 200 μm; C: Dorsal view of cephalon with rather slender neck; scale 100 μm; D: Right 3rd leg; scale 100 μm; E: Tarsus, strongly curved propodus, and claw, auxiliary claws about half as long as claw (right 3rd leg); scale 100 μm; F: Segment 3, 4 and abdomen; scale 40 μm.
Figure 29. Callipallene emaciata, female; A: Dorsal view; scale 1 mm; B: Frontal view; scale 1 mm; C: Dorsal view of trunk; scale 200 µm; D: Dorsal view of cephalon with rather slender neck; scale 200 µm; E: Left chela, dorsal is up; scale 40 µm; F: Tarsus, strongly curved propodus, and claw, auxiliary claws about half as long as claw (left 3rd leg); scale 100 µm.
Figure 30. *Callipallene phantoma*, male; A: Dorsal view of trunk; scale 200 µm; B: Ventral view of trunk; scale 200 µm; C: Dorsal view of cephalon with very long and slender neck; scale 100 µm; D: Ventral view of proboscis; scale 100 µm; E: Left chela, dorsal is down; scale 40 µm; F: Left 10-articled oviger; scale 100 µm.
Figure 31. *Callipallene phantoma*, male; A: Distal articles of left oviger with compound spines; scale 40 µm; B: Compound spines on last oviger-article; scale 5 µm; C: Left 3rd leg; scale 200 µm; D: Ventral view of coxa 2 with genital opening, distal is down (right 2nd leg); scale 20 µm; E: Genital opening; scale 5 µm; F: Tarsus, straight propodus, and claw with very short auxiliary claws (right 3rd leg); scale 40 µm; G: Hair and slit organ on dorsal side of trunk; scale 5 µm.
Figure 32. *Callipallene phantoma*, female; A: Dorsal view of trunk; scale 200 µm; B: Ventral view of trunk; scale 200 µm; C: Dorsal view of cephalon with very long and slender neck; scale 200 µm; D: Frontal view of cephalon; scale 100 µm; E: Mouth opening; scale 20 µm; F: Left chela, dorsal is up; scale 40 µm.
Figure 33. *Callipallene phantoma*, female; A: Right 10-articled oviger; scale 100 µm; B: Distalmost oviger-articles with compound spines; scale 20 µm; C: Right 3rd leg; scale 200 µm; D: Ventral view of coxa 2 with genital opening, distal is down (left 4th leg); scale 40 µm; E: Genital opening; scale 10 µm; F: Tarsus, straight propodus, and claw with very short auxiliary claws (left 3rd leg); scale 40 µm; G: Small auxiliary claws; scale 10 µm.
Figure 34. *Callipallene producta*, female; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk; scale 200 µm; C: Ventral view of trunk; scale 200 µm; D: Dorsal view of cephalon with very long and slender neck; scale 200 µm; E: Ventral view of proboscis; scale 100 µm; F: Ocular tubercle with lateral sense organ; scale 40 µm.
Figure 35. *Callipallene producta*, female; **A**: Distal article of right 10-articled oviger; scale 40 μm; **B**: Compound spines on oviger-article 7; scale 10 μm; **C**: Right 3rd leg; scale 400 μm; **D**: Ventral view of coxa 2 with genital opening, distal is down (left 3rd leg); scale 40 μm; **E**: Genital opening; scale 10 μm; **F**: Tarsus, slightly curved propodus, and claw with very long auxiliary claws (right 3rd leg); scale 100 μm; **G**: Claw and auxiliary claws; scale 40 μm.
Figure 36. *Callipallene spectrum*, male; A: Dorsal view of trunk; scale 100 µm; B: Lateral view of trunk; scale 100 µm; C: Dorsal view of cephalon; scale 100 µm; D: Cephalon with proboscis and chelifore; scale 100 µm; E: Left 3rd leg; scale 200 µm; F: Tibia2, tarsus, propodus, and claws (left 3rd leg); scale 100 µm.
Figure 37. Callipallene tiberi, female; A: Dorsal view of trunk; scale 200 µm; B: Dorsal view of cephalon with very short neck; scale 100 µm; C: Mouth opening; scale 20 µm; D: Left 3rd leg; scale 200 µm; E: Tarsus, strongly curved propodus, and claw, auxiliary claws about half as long as claw (left 3rd leg); scale 100 µm; F: Segment 3, 4 and abdomen; scale 40 µm.
Figure 38. Endeidae; A, B: *Endeis charybdae*, male, dorsal view; scales 3 mm and 1 mm, respectively; C, D: *Endeis spinosa*, male, dorsal view; scales 1 mm and 500 µm, respectively.
Figure 39. *Endeis charybdea*, male; A: Dorsal view of trunk; scale 1 mm; B: Dorsal view of proboscis; scale 200 µm; C: Tip of proboscis with numerous spines around mouth; scale 100 µm; D: Ocular tubercle; scale 100 µm; E: 7-articled oviger; scale 200 µm; F: Distal articles of oviger; scale 100 µm.
Figure 40. *Endeis charybdae*, male; A: Right lateral process 1 with 2 protuberances; scale 100 µm; B: Coxa 1 of right 1st leg with 2 protuberances; scale 100 µm; C: Right 3rd leg; scale 1 mm; D: Femur with 8 of overall 24 cement glands (left 3rd leg); scale 100 µm; E: Single cement gland; scale 5 µm; F: Tarsus, propodus, and claws (right 3rd leg); scale 200 µm; G: Abdomen; scale 200 µm.
Figure 41. 

*Endeis spinosa*, male; 

A: Dorsal view, abdomen lost due to preparation; scale 1 mm; 

B: Dorsal view of trunk; scale 400 µm; 

C: Dorsal view of proboscis; scale 200 µm; 

D: Tip of proboscis with few spines around mouth; scale 100 µm; 

E: Mouth opening; scale 20 µm; 

F: Cephalon with small protuberances with spine and without chelifores; scale 40 µm.
Figure 42. *Endeis spinosa*, male; **A**: Ocular tubercle; scale 100 µm; **B**: 7-articled oviger; scale 200 µm; **C**: Small spine on oviger-article 6; scale 10 µm; **D**: Right lateral process 1 with 2 protuberances; scale 100 µm; **E**: Right lateral process 3 with 1 protuberance; scale 100 µm; **F**: Coxa 1 of right 1st leg with 2 protuberances; scale 100 µm; **G**: Coxa 1 of right 3rd leg with 1 protuberance; scale 100 µm; **H**: Left 3rd leg; scale 400 µm.
Figure 43. *Endeis spinosa*, male; A: Lateral view of coxa 2 with genital opening on a protuberance (right 3rd leg); scale 100 µm; B: Genital opening; scale 20 µm; C: Femur with overall about 11 cement glands (left 3rd leg); scale 200 µm; D: Single cement gland; scale 10 µm; E: Tarsus, propodus, and claws (left 3rd leg); scale 200 µm; F: Spine, hair and slit organ on dorsal side of trunk; scale 10 µm.
Figure 44. *Endeis spinosa*, female; A: Dorsal view of trunk; scale 1 mm; B: Ocular tubercle; scale 100 µm; C: Right lateral process 3 without protuberance; scale 100 µm; D: Coxa 1 of right 3rd leg without protuberance; scale 100 µm; E: Right 3rd leg; scale 1 mm; F: Genital opening on ventral side of coxa 2; scale 40 µm; G: Abdomen; scale 200 µm.
Figure 45. Phoxichilidiidae; A, B: *Anoplodactylus angulatus*, female, dorsal view; scales 500 µm and 250 µm, respectively; C, D: *Anoplodactylus petiolatus*, male, dorsal view; scales 1 mm and 250 µm, respectively; E, F: *Anoplodactylus pygmaeus*, male, dorsal view; scales 1 mm and 250 µm, respectively.
Figure 46 *Anoplodactylus angulatus*, female; **A:** Dorsal view; scale 400 µm; **B:** Lateral view; scale 400 µm; **C:** Dorsal view of trunk; scale 200 µm; **D:** Ventral view of trunk; scale 100 µm; **E:** Frontal view with chelifores with developed chela and mouth opening; scale 40 µm; **F:** Mouth opening; scale 20 µm.
Figure 47. *Anoplodactylus angulatus*, female; A: Ventral view of proboscis with conspicuous angles at corners (arrowheads); scale 40 µm; B: Ocular tubercle; scale 40 µm; C: Right lateral process 3 with spine; scale 40 µm; D: Right 3rd leg; scale 200 µm; E: Tarsus and propodus with hollow spine on propodus (right 3rd leg); scale 40 µm; F: Hollow spine on propodus with slit on tip (arrowhead); scale 5 µm.
Figure 48. *Anoplodactylus petiolatus*, male; A: Dorsal view of trunk; scale 200 µm; B: Lateral view of cephalon overreaching posterior part of proboscis, oviger dissected; scale 200 µm; C: Chelifores with developed chela; scale 40 µm; D: Left lateral process 3 with protuberance; scale 40 µm; E: Tarsus, propodus with cutting lamina, and claws (left 3rd leg); scale 40 µm; F: Cutting lamina on propodus (right 1st leg); scale 20 µm.
Figure 49. Anoplodactylus pygmaeus, male; A: Dorsal view; scale 400 µm; B: Lateral view of trunk; scale 200 µm; C: Dorsal view of trunk; scale 200 µm; D: Ventral view of trunk; scale 200 µm; E: Lateral view of cephalon overreaching posterior part of proboscis, proboscis with constriction encircling it subterminally (arrowheads); scale 100 µm; F: Ventral view of proboscis with constriction encircling it subterminally (arrowheads); scale 40 µm.
Figure 50. *Anoplodactylus pygmaeus*, male; A: Mouth opening; scale 20 µm; B: Left chelifore with developed chela; scale 20 µm; C: Ventral view of right palp, reduced to a small tubercle; scale 40 µm; D: Ocular tubercle with lateral sense organ; scale 40 µm; E: 6-articled oviger; scale 100 µm; F: Distal articles of left oviger; scale 20 µm.
Figure 51. *Anoplodactylus pygmaeus*, male; A: Right lateral process 3 with protuberance and spine; scale 20 µm; B: Left 3rd leg; scale 200 µm; C: Ventral view of coxa 2 with genital opening, distal is down (left 4th leg); scale 20 µm; D: Dorsal view of femur with cement gland, distal is down (right 4th leg); scale 20 µm; E: Tarsus, propodus with cutting lamina, and claws (left 3rd leg); scale 100 µm; F: Cutting lamina on propodus; scale 100 µm; insert: Detail of cutting lamina; scale 20 µm; G: Hairs and slit organs on dorsal side of trunk; scale 10 µm.
Figure 52. *Anoplodactylus pygmaeus*, female; A: Dorsal view of trunk; scale 200 µm; B: Ventral view of trunk, proboscis with a constriction encircling it subterminally (arrowheads); scale 200 µm; C: Right lateral process 3 with protuberance and spine; scale 40 µm; D: Ventral view of coxa 2 with genital opening (right 4th leg); scale 40 µm; E: Genital opening; scale 10 µm; F: Tarsus, propodus with cutting lamina, and claws (left 1st leg); scale 40 µm; G: Cutting lamina on propodus; scale 5 µm.
Figure 53. Pycnogonidae; A, B: *Pycnogonum nodulosum*, female, dorsal view; scales 1 mm and 500 µm, respectively; C, D: *Pycnogonum (Retroviger) pusillum*, male, dorsal and ventral view, respectively; scales 250 µm.
Figure 54. *Pycnogonum nodulosum*, female; A: Dorsal view; scale 1 mm; B: Dorsal view of trunk with protuberances on dorsal midline; scale 1 mm; C: Lateral view of trunk; scale 1 mm; D: Left 3rd leg with protuberances on femur; scale 400 µm; insert: Dorsal view of protuberances on femur; scale 200 µm; E: Tarsus, propodus, and claw without auxiliary claws (left 3rd leg); scale 100 µm; insert: Spines on propodus; scale 20 µm; F: Hair and slit organ on dorsal side of trunk; scale 5 µm.
Figure 55. *Pycnogonum (Retroviger) pusillum*, male; A: Dorsal view; scale 400 µm; B: Dorsal view of proboscis; scale 100 µm; C: Right 3rd leg; scale 100 µm; D: Tarsus, propodus, and claw without auxiliary claws (right 3rd leg); scale 40 µm; E: Abdomen; scale 100 µm; F: Protuberance, scattered over trunk and legs; scale 5 µm.
Acknowledgements

We thank Stefan Friedrich for expert technical assistance; Katharina Jörger and Roland Meyer for help with collecting the animals; and Stefan Füssl for help with preparing the figures in Adobe Photoshop.

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A redescription of the type species of *Oedicerina* Stephensen, 1931 (Crustacea, Amphipoda, Oedicerotidae) and the description of two new species

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Abstract

The poorly known species *Oedicerina ingolfi* Stephensen, 1931 (Crustacea: Amphipoda: Oedicerotidae) is redescribed, based on new material from the Norwegian Sea. *Oedicerina vaderi* sp. n. from the northeast Atlantic Ocean and *Oedicerina loerzae* sp. n. from New Zealand waters are described raising the number of species in the genus to five. The three species treated here together with *Oedicerina megalopoda* Ledoyer, 1986 and *Oedicerina denticulata* Hendrycks & Conlan, 2003 are separated by characters of the rostrum, maxilliped, gnathopods, epimera, and by the dorsal armature of pleonites and urosomites. The genus is recorded from the Atlantic, Indian and Pacific Oceans, mainly at bathyal depths.

Key Words

Taxonomy
Amphipoda
Oedicerotidae
*Oedicerina*
new species
Norwegian Sea
North Atlantic Ocean
New Zealand
Pacific Ocean

Introduction

Participants in a workshop organised by Professor Wim Vader at the University of Tromsø field station at Skibotn in 2009 sorted and identified amphipods from the Natural History Collections of the University Museum of Bergen collected from the Norwegian Sea. Among the extensive material in these collections were specimens of the poorly known oedicerotid genus *Oedicerina* Stephensen, 1931. The genus was based on a single individual collected from the Norwegian Sea by The Danish-Ingolf Expedition (Stephensen 1931). Although the specimen consisted of just the head and pereonites 1–4 and was lacking gnathopods, the new genus was created for *O. ingolfi* Stephensen, 1931 to recognise the unique nature of the huge posterior lobe of coxa 4. The genus remained monotypic and unreported until Ledoyer (1986) described *Oedicerina megalopoda* from the Mozambique Channel, western Indian Ocean, also based on a single anterior fragment. This species was defined by the characteristic shape of coxa 4 and by the strongly developed carpal articles of gnathopods 1 and 2. The description of a third species, *O. denticulata* Hendrycks & Conlan, 2003 from the northeast Pacific Ocean, was the first to record complete specimens and was accompanied by a more detailed appraisal of the genus.

The new material from the Bergen Museum has made possible a re-description of *O. ingolfi*. Specimens belonging to *Oedicerina* from the northeast Atlantic Ocean and
from New Zealand waters demonstrate further diversity within the genus and require the recognition of two new species which are described below.

This paper is the third to utilize material sorted at Skibotn in 2009, following d’Udekem d’Acoz (2010) and Krapp-Schickel and Vader (2013).

**Material and methods**

Norwegian Sea material assigned to *O. ingolfi* from the Natural History Collection of the University Museum of Bergen (ZMBN) was collected with an RP sledge (Rothlisberg and Pearcy 1977) by Torleiv Brattegard (Brattegard and Fossà 1991) in the period 1981–1986. Additional material from the Norwegian and Greenland Seas from the Museum of Zoology, Lund University was collected during the NORBI expedition (Dahl et al. 1976) using an epibenthic sledge (drague Sanders) (Guennegan and Martin 1985).

Specimens from the Discovery Collections at the National Oceanography Centre, Southampton were obtained in the East Iceland Basin on an RRS *Discovery* cruise that contributed to the Institute of Oceanographic Sciences investigations of mid-water and benthic faunas in the eastern North Atlantic Ocean (1965–1977). The material, from an epibenthid sledge, was fixed in 4% formaldehyde and later transferred to 70% Industrial Methylated Spirits. These specimens have been deposited in the Amphipoda collections at The Natural History Museum, London.

The New Zealand material was collected during the Ocean Survey 2020 expeditions with RV *Tangaroa* to the Chatham Rise and the Challenger Plateau (Knox et al. 2012) by means of a “Brenke” epibenthic sledge (Brenke 2005). The material was sorted on board, fixed in 96% ethanol and later transferred into 70% ethanol. It has been deposited in the National Institute of Water and Atmosphere Research (NIWA) Marine Invertebrate Collection in Wellington, New Zealand.

For habitus drawings the specimens were transferred into glycerol on a cavity slide. Specimens were then dissected under a stereomicroscope (Leica M205 or Wild M5) using dissecting needles. Mouthparts and appendages were mounted temporarily in glycerol on slides for microscopic examination and drawing. Appendages were later mounted as permanent slides with glycerol jelly, or temporary mounts using Polyvinyl-lactophenol stained with lignin-pink. Drawings of habitus and appendages were made using a *camera lucida* attached to a compound microscope (Leica DMLB or Wild M20). Pencil drawings were scanned, inked digitally and arranged to plates using the methods described in Coleman (2003, 2009).

Body lengths were measured along the dorsal outline from the tip of the rostrum to the end of the telson. Lengths of individual articles of gnathopods and pereopods measured along anterior or posterior margins can vary depending on the degree of flexure of the appendage. All articulations except those between coxae and tergites and between merus and carpus of gnathopods are bicondylar. Measurements made between condyles gives a length that is not affected by limb flexure. Length ratios herein have been derived using this principle.

**Systematics**

Amphipoda Latreille, 1816
Oedicerotidae Lilljeborg, 1865

*Oedicerina* Stephensen, 1931

*Oedicerina* Stephensen, 1931: 250. — Barnard and Karaman 1991: 561, — Ledoyer 1986: 832. — Hendrycks and Conlan 2003: 2359.

**Diagnosis (key characters embolded).** Rostrum well-developed, moderately to strongly deflexed. Antennae sexually dimorphic or not, length medium. Antenna 1 about as long as head and pereonites 1–4 combined, peduncle article 1 longer than articles 2 and 3. Antenna 2 subequal to or weakly longer than antenna 1; peduncle article 4 longer than article 5. Lower lip, inner lobes prominent, separate. Mandible, molar triturative; incisor 5-dentate. Maxilla 1, outer plate 9-dentate; palp slender, article 2 subequal to or longer than article 1. Maxilla 2, plates short, inner broader than outer. Maxilliped, palp article 2 sub-triangular, breadth greatest at half-length, inner margin strongly convex; article 3 produced mediodistally; article 4 longer than article 3.

Coxal plates 1–4 deep, as long or longer than height of corresponding pereonite. Gnathopod 1, coxa expanded distally; carpus and propodus subequal in length, strongly expanded posterodistally. Gnathopod 2, carpus longer than propodus, both strongly expanded posterodistally. Pereopods 3 and 4 fossorial (setose); coxa 4 deeply excavate posteriorly, posterodistal lobe strong, subrectangular. Pereopod 5, coxa bilobate, posterior lobe as long as coxa 4. Pereopod 6, coxa bilobate, posterior lobe strong. Pereopod 7, basis expanded.

Pleonites, some or all carinate or toothed. Epimera 1–3, 1 and 3 rounded, 2 obtusely rounded, posterior margin convex or sinuous. Uropods 1–2, outer ramus subequal to or shorter than inner ramus. Uropod 3, peduncle short; rami subequal, not extending as far as apices of uropods 1–2. Telson notched 30–40%, apices acute.

**Type species.** *Oedicerina ingolfi* Stephensen, 1931

**Species composition.** *Oedicerina denticulata* Hendrycks & Conlan, 2003; *Oedicerina ingolfi* Stephensen, 1931; *Oedicerina loerzei* sp. n.; *Oedicerina megalopoda* Ledoyer, 1986; *Oedicerina vaderi* sp. n.
Key to the species of Oedicerina

1 Rostrum massive, spatulate, longer than peduncle article 1 of antenna 1 .................. O. megalopoda Ledoyer, 1986
- Rostrum at most as long as article 1 of antenna 1 .................................................................................. 2
2 Pleonites 1–2 carinate, pleonite 3 smooth .............................................................................................. 3
- Some pleonites smooth dorsally ........................................................................................................... 3
3 Pleonite 1 smooth .................................................................................................................................. 4
- Pleonite 1–2 carinate, pleonite 3 smooth .............................................................................................. 4
4 Pleonite 1–2 smooth ............................................................................................................................... 4
- Pleonite 2 carinate, pleonite 3 with short process ............................................................................... 4

Oedicerina ingolfi Stephensen, 1931

Figs 1–5

Oedicerina ingolfi Stephensen, 1931, p. 250, fig. 72. Oedicerinidae gen. et sp. n. Dahl 1979, p. 60 (ecology).

Material examined. 3 ovig. females, 6 females, 3 males, 2 unknown sex, 4 juveniles, ZMBN 95143, St. 81.08.14.5, 64°16.9’N, 00°11.7’W, 2630 m, F/F Håkon Mosby, RP-sledge, T. Brattegard, 14 August 1981.

1 female, 1 male, 1 juvenile, ZMBN 95144, St. 81.08.14.1, 65°19.7’N 01°02.7’E, 2908 m, F/F Håkon Mosby, RP-sledge, T. Brattegard, 14 August 1981.

1 ovig. female, 4 females, 7 males, 3 unknown sex, 12 juveniles, ZMBN 95145, St. 82.11.24.1 64°48.2’N 01°33.0’W, 3000 m, F/F Håkon Mosby, RP-sledge, T. Brattegard, 24 November 1982.

1 male, 1 juvenile, ZMBN 95146, St. 81.06.03.5, 67°47.0’N 07°43.9’E, 2025 m, F/F Håkon Mosby, RP-sledge, T. Brattegard, 3 June 1981.

1 female, ZMBN 95147, St. 86.07.26.1, 69°36.4’N 09°54.6’W; 2212 m, F/F Håkon Mosby, RP-sledge, T. Brattegard, 26 July 1986.

2 adult females, 1 female, 2 juveniles, NORBI St. 2, DS05, 65°22.9’N 00°02.1’E–65°22.4’N 00°02.2’E; 2970 m, N.O. Jean Charcot, 21 July 1975.

1 ovig. female, 1 adult male, NORBI St. 6, DS12, 76°54.4’N 01°44.6’E–76°54.0’N 01°46.3’E; 3200 m, N.O. Jean Charcot, 2 August 1975.

Description. Based on ovigerous female, 10.3 mm, St. 81.08.14.5.

Head (Fig. 1a): longer than high, longer than pereonites 1–2 combined; no eyes or ocular pigment visible; rostrum strongly deflexed, ventral margin weakly convex. Antenna 1 (Fig. 1b): about as long as antenna 2; length ratios of peduncle articles 1–3: 1:0.9:0.6; flagellum 10-articulate; accessory flagellum 1-articulate, minute, slender, less than half length of first flagellum article. Antenna 2 (Fig. 1c): peduncle setose; length of article 4 1.6 × article 5; flagellum shorter than peduncle article 5, 7-articulate. Upper lip (labrum) (Fig. 1d): wider than long, rounded apically. Mandible: molar triturative, with one associated seta; incisors and laciniae mobiles 5-dentate; palp (Fig. 1i) 3-articulate, article 2 swollen proximally, article 3 tapered, length ratios of articles 1–3 1:4:1.4.5. Lower lip: inner lobes prominent and broad, hypopharyngeal gap wide, outer lobe mandibular processes short and rounded. Maxilla 1 (Fig. 1e, f, g): inner plate oval, with two distal setae; outer plate with nine acute setal-teeth; palp 2-articulate, article 2 3.6 × length of article 1. Maxilla 2 (Fig. 2a): inner plate 1.2 × wider than outer plate; both plates with relatively sparse apical setation. Maxilliped (Fig. 2b): inner plate short, extending just beyond base of palp article 1; outer plate extending 50% along palp article 2, concave medially; palp 4-articulate; article 1 tapered; article 2 broad, strongly expanded medially, lobe subtriangular; article 3 narrow proximally, expanded mediostally; article 4 acute, weakly falcate; length ratios of articles 1–4 1:1.7:0.7:1.3.

Pereon. Pereonite 1 (Fig. 1a): longer than 2; pereonites 3–5 successively longer; pereonites 6 and 7 subequal in length to 5. Gnathopod 1 (Fig. 2c): coxa subtriangular, posterior margin straight, anterodistal corner rounded, posterodistal corner rectangular, distal margin straight, strongly setose; basis straight, weakly expanded, posterior margin with a row of plumose setae; merus, posterodistal lobe rounded, setose; carpus strongly expanded, subacute posterior lobe with posterior and distal margins setose; propodus strongly expanded, as long and wide as carpus, anterior margin convex, palm transverse, convex, crenellate and setose; dactylus curved, just longer than palm. Gnathopod 2 (Fig. 3a): coxa as long as coxa 1, weakly tapering distally, apex rounded, setose; basis subrectangular, with a row of plumose setae near posterior margin; merus, postero-distal lobe narrow, setose; carpus strongly expanded, wider than propodus, postero-distal lobe subacute, distal margin oblique; propodus shorter than carpus, expanded distally, palm strongly convex, crenellate; dactylus slender, falcate, as long as palm. Pereopod 3 (Fig. 3b): coxa subequal to coxa 2; basis shorter than coxa, with very long slender setae on posterior margin and plumose setae close to anterior margin; merus weakly expanded distally; carpus 1.3 × length and about as wide as merus, posterior margin setose; propodus oval, setose; dactylus 1.2 × length of propodus. Pereopod 4 (Fig. 4a): coxa wider than long, distal margin rounded, postero-distal lobe very strong, subrectangular; basis shorter than coxa; merus weakly expanded; carpus shorter than merus, but subequal in width, setose posteriorly and anterodistally; propodus with anteromarginal rows of slender setae; dactylus rather stout, short and straight. Pereopod 5 (Fig. 4b): coxa as deep as coxa 4, bilobed, posterior lobe

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Figure 1. *Oedicerina ingolfi*, ovig. female, 10.3 mm; Norwegian Sea, ZMBN 95143, St. 81.08.14.5. a) habitus (uropods rolled up, not illustrated); b) antenna 1; c) antenna 2; d) upper lip; e) maxilla 1; f) maxilla 1, setal teeth of outer plate; g) maxilla 1, distal setation of palp article 2; h) telson; i) mandibular palp. Scale bars: a; 2 mm: b, c, e, h; 200 μm: d, i; 100 μm.
Figure 2. *Oedicerina ingolfi*, ovig. female, 10.3 mm; Norwegian Sea, ZMBN 95143, St. 81.08.14.5. a) maxilla 2; b) maxillipeds; c) gnathopod 1. Scale bars: a; 100 µm; b, c; 200 µm.
Figure 3. Oedicerina ingolfi, ovig. female, 10.3 mm; Norwegian Sea, ZMBN 95143, St. 81.08.14.5. a) gnathopod 2; b) pereopod 3. Scale bars: a, b; 200 µm.
Figure 4. *Oedicerina ingolf*, ovig. female, 10.3 mm; Norwegian Sea, ZMBN 95143, St. 81.08.14.5. a) pereopod 4; b) pereopod 5. Scale bars: a, b; 200 µm.
Figure 5. *Oedicerina ingolfi*, ovig. female, 10.3 mm; Norwegian Sea, ZMBN 95143, St. 81.08.14.5. a, d) pereopod 6; b) pereopod 7; c) pleopod 1; e) uropod 1; f) uropod 2; g) uropod 3. Scale bars: a, b; 1 mm: c, e, f, g; 200 µm.
specimens of all sizes, both female and male, and was collected relatively close to the type locality of *O. ingolfi*, but nevertheless we cannot be absolutely sure that our material represents Stephensen’s species (see discussion below).

### Oedicerina vaderi sp. n.

http://zoobank.org/0CBB4731-9908-43F5-83E5-BCF9C83ED4C6

**Type material.** Male holotype, 7.3 mm; NHMUK 2014. 398, Discovery Stn 7709#73.

**Type locality.** North Atlantic, East Iceland Basin: 60°07.1’N 19°30.3’W – 60°06.1’N 19°24.8’W, 5 May 1971, BN 2.4, 2636–2646 m.

**Paratypes.** 1 female, 6.3 mm; 2 specimens of unknown sex, 4.2 mm and 5 mm; NHMUK 2014. 399-401, Discovery Stn 7709#73, from the type locality.

**Etymology.** The specific name *vaderi* recognises the important contributions to amphipod studies made by Professor Wim Vader.

**Description.** Holotype male, 7.3 mm. **Head** (Fig. 6c): longer than high, longer than pereonites 1–2 combined; no eyes or ocular pigment visible; rostrum (Fig. 6c) strongly deflexed, the ventral margin concave. **Antenna 1** (Fig. 6f): length ratios of peduncle articles 1–3 1:0.5:0.3; flagellum [broken], proximal flagellum articles wider than long; accessory flagellum 1-articulate, slender, about half the length of peduncle article 3. **Antenna 2** (Fig. 6g): peduncle weakly setose; length of article 4 1.5 × article 5; flagellum shorter than peduncle article 5, 9-articulate. **Upper lip** (labrum) (Fig. 6d): wider than long, apically rounded. **Mandible** (Fig. 7a, c): incisors and laciniae mobiles 5-dentate; palp 3-articulate, article 2 swollen proximally, article 3 tapered, length ratios of articles 1–3 1:4.3:6.1. **Lower lip**; inner lobes prominent and broad, hypopharyngeal gap wide, outer lobe mandibular processes short and rounded. **Maxilla 1** (Fig. 7d): inner plate tapered, with two distal setae; outer plate with nine acute setal-teeth; palp 2-articulate, article 2.5 × length of article 1. **Maxilla 2** (Fig. 7b): inner plate 1.3 × wider than outer plate; both plates with relatively sparse apical setation. **Maxilliped** (Fig. 7c): inner plate short, extending just beyond base of palp article 1; outer plate extending 30% along palp article 2; concave medially; palp 4-articulate; article 1 tapered; article 2 broad, strongly expanded medially, lobe broadly rounded; article 3 narrow proximally and expanded mediiodistally; article 4 curved, acute; length ratios of articles 1–4 1:2.2:0.8:1.3.

**Pereon.** **Pereonite 1** (Fig. 6a): longer than 2; pereonite 2 shortest. **Gnathopod 1** (Fig. 8a): coxa subtriangular, posterior margin straight, anterodistal corner broadly rounded, posterodistal corner rectangular, distal margin straight, weakly setose; basis curved, posterior margin with a row of plumose setae; merus, posterodistal lobe rounded, setose; carpus strongly expanded, subrectangular posterior.
Coleman, C. O. & Thurston, M. H.: A redescription of the type species of *Oedicerina*

Figure 6. a, c–g) *Oedicerina vaderi* sp. n., male holotype, 7.3 mm; northeast Atlantic Ocean, NHMUK 2014. 398, Discovery Stn 7709/#73. a) habitus; c) head; d) upper lip; e) epimeral plates 1–3; f) antenna 1; g) antenna 2. Female paratype, 6.3 mm; same locality. b) antenna 1. Scale bars: a; 1 mm: b–g; 200 μm.
lobe with posterior and distal margins setose; propodus strongly expanded, as long and as wide as carpus, anterior margin convex, palm convex, transverse, crenellate, setose; dactylus falcate, as long as palm. 

Gnathopod 2 (Fig. 8b): coxa as long as coxa 1, tapering distally, apex truncate with few setae at the distal margin; basis subrectangular, with a posterodistal group of plumose setae and an anterodistal group of simple setae; merus, posterodistal lobe narrow, setose; carpus strongly expanded, wider than propodus, posterodistal lobe subacute, posterior and distal margins setose; propodus as long as carpus, expanded distally, palm convex, crenellate; dactylus slender, curved, as
Figure 8. *Oedicerina vaderi* sp. n., male holotype, 7.3 mm; northeast Atlantic Ocean, NHMUK 2014. 398, Discovery Stn 7709#73. 

a) gnathopod 1; b) gnathopod 2. Scale bars: a, b; 200 μm.
long as palm. *Pereopod 3* (Fig. 9a): coxa subequal to coxa 2, apex rounded, weakly setose; basis shorter than coxa, long plumose setae distally along posterior and anterior margins; merus expanded anterodistally, setose; carpus 1.3 × length and about as wide as merus, posterior margin densely setose; propodus oval, anterodistal and posterior margins setose; dactylus lanceolate, subequal to propodus. *Pereopod 4* (Fig. 9b): coxa wider than long, anterior mar-
Figure 10. a–d, f) Oedicerina vaderi sp. n., male holotype, 7.3 mm; northeast Atlantic Ocean, NHMUK 2014. 398, Discovery Stn 7709#73. a) pereopod 6; b) pereopod 7; c) uropod 3; d) uropod 2, peduncle; f) telson; g) uropod 1. Female paratype, 6.3 mm; same locality. e) pleonite 3 and urosome with telson, uropods not shown, Scale bars: a–d; 200 μm: e; 1 mm.
gin broadly rounded, distal margin straight, posterodistal lobe very strong, subrectangular; basis shorter than coxa, anterior and posterior margins setose distally; merus expanded anterodistally, setose; carpus 0.8 × merus, but sub-equal in width, posterior margin strongly setose, long setae anterodistally; propodus, anterior margin setose; dactylus, straight, lanceolate, 1.5 × propodus. Pereopod 5 (Fig. 9c): coxa about as deep as coxa 4, bilobed, posterior lobe expanded distally, distal margin straight, anterior lobe 0.7 × length of posterior lobe, rounded distally; basis shorter than coxa; merus as long as basis; propodus 0.3 × length of merus; propodus slender, subrectangular, 0.9 × merus, about as long as straight lanceolate dactylus; articles 2–6 variously setose. Pereopod 6 (Fig. 10a): coxa 0.8 × length of coxa 5, bilobed, posterior lobe long, distal margin rounded, anterior lobe subrectangular, 0.4 × length of posterior lobe; basis subrectangular; merus, posterior margin weakly convex; carpus subrectangular, 0.4 × length of merus; propodus and straight dactylus as long as merus; articles 2–6 variously setose. Pereopod 7 (Fig. 10b): long; coxa wider than long, oval, posterodistal corner rounded; basis posterior margin weakly convex, anterior margin strongly convex; merus elongate; carpus, 0.9 × merus; merus and carpus with groups of short setae on anterior and posterior margins; [propodus and dactylus unknown].

**Pleon.** Pleonites 1–3 (Fig. 6a): smooth, lacking carinæ or teeth. Epimera (Fig. 6e): 1 and 3 evenly rounded; epimeron 2, posterodistal angle produced, rounded, posterior margin sinuous. Pleopod 1: peduncle stout, 0.8 × length of rami; rami subequal in length. Urosome. Urosomites 1 (Fig. 10e): longest, with low carapace, posterodistal angle produced, rounded, posterior margin sinuous. Pleopod 1: peduncle elongate, lateral margin with robust setae, mesial margin setose; inner ramus, [broken], both margins setose; outer ramus 0.7 × length of peduncle, setae on lateral margin only. Uropod 2 (Fig. 10d): peduncle not tapering, both margins with short setae; [rami damaged], inner ramus, both margins setose; outer ramus, lateral margin setose. Uropod 3 (Fig. 10c): peduncle short, about as long as telson, with ventral subacute projection; outer ramus just longer than inner ramus. Telson (Fig. 10f) tapered, notched 40%.

**Variability.** The paratypes bear a small posteriorly directed tooth on pleonite 3. It may be that this process has been present and is worn down in the holotype. Antenna 1 of the female (Fig. 6b) has a longer and more slender peduncle and fewer and more elongate flagellum articles compared to the male.

**Distribution.** North Atlantic, south of Iceland, 2636–2646 m.

**Oedicerina loerzei** sp. n.

http://zoobank.org/34665247-D04C-4354-9314-13E38286AA04 Figs 11–16

**Holotype.** Male, 8.5 mm; NIWA 84727, TAN 0705-41, Chatham Rise, 43°50'10.8"S 176°42'33.0"E, 478–479 m, 5 April 2007. **Paratypes.** Male?, 7.7 mm; NIWA 89970, TAN 0705-251, Chatham Rise, 42°59'45.0"S 178°59'44.4"E, 520–530 m, 24 April 2007; ovig. female, 7 mm; NIWA 84740, TAN0705-83, Chatham Rise, 43°50'10.8"S 176°42'33.0"E, 529–530 m; 9 April 2007.

**Etymology.** The species is named for Dr. Anne-Nina Lörz to acknowledge her significant contributions to amphipod systematics.

**Description.** Holotype male, 8.5 mm. **Head** (Fig. 11a): longer than high, somewhat longer than pereonites 1–2 combined; no eyes or ocular pigment visible; rostrum (Fig. 11c) strongly deflexed, the ventral margin weakly convex. **Antenna 1** (Fig. 12a): shorter than antenna 2; length ratios of peduncle articles 1–3 1:0.5:0.3; flagellum 19-articulate, first article as long as peduncle article 3, proximal articles wider than long; accessory flagellum 1-articulate, minute, 0.3 × length of primary flagellum article 1. **Antenna 2** (Fig. 12c): peduncle setose; length of article 4 1.2 × article 5; flagellum 23-articulate, 1.9 × length of peduncle article 5. **Upper lip** (labrum) (Fig. 11b): wider than long, truncate apically. **Mandible** (Fig. 12b, d): incisor 5-dentate; left lacinia mobilis wide and multidentate, right narrower; palp 3-articulate, article 3 tapered, length ratios articles 1–3 1:3.9:4.9. **Lower lip** (Fig. 11d): inner lobes short and broad, hypopharyngeal gap wide, outer lobe mandibular processes acute. **Maxilla 1** (Fig. 11e): inner plate tapered, with one distal seta; outer plate with nine acute setal-teeth; palp 2-articulate, article 3 expanded, length ratios articles 1–3 1:1.7:1.7:1.2. **Maxilliped** (Fig. 13a): outer plate with nine acute setal-teeth, acute, subtriangular, strongly expanded distally, anterior and posterior margins setose; propodus strongly expanded distally, as wide and as long as carpus, anterior and posterior margins convex, palm transverse, convex, crenelate, setose; dactylus slightly curved, just longer than palm. **Maxilliped** (Fig. 13a): outer plate with nine acute setal-teeth, acute, subtriangular, strongly expanded distally, anterior and posterior margins setose; propodus strongly expanded distally, as wide and as long as carpus, anterior and posterior margins subparallel, apex truncate, weakly setose; basis a little expanded, with some plumose setae near posterior and distal margins and an
Figure 11. *Oedicerina loerzae* sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. a) habitus; b) upper lip; c) rostrum; d) lower lip; e) maxilla 1. Scale bars: a; 1 mm: b–e; 100 μm.
Figure 12. *Oedicerina loerzae* sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. a) antenna 1; b) left mandible; c) antenna 2; d) right mandible; e) maxilliped, outer plate; f) maxilla 2; g) maxilliped, inner plate. Scale bars: a, b, d–g; 100 μm: c; 200 μm.
Figure 13. Oedicerina loerzae sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. **a)** outline of maxillipeds; **b)** maxilliped palp; **c)** gnathopod 1. Scale bars: a–c; 200 μm.
Figure 14. *Oedicerina loerzae* sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. a) gnathopod 2; b) gnathopod 2, detail of palm; c) pereopod 3, setation of carpus and propodus omitted; d) pereopod 4. Scale bars: a, c, d; 200 µm.
Figure 15. *Oedicerina loerzae* sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. a) pereopod 5, b) pereopod 7; c) pereopod 6. Scale bars: a–c; 200 μm.
Figure 16. *Oedicerina loerzae* sp. n., male holotype, 8.5 mm; southwest Pacific Ocean, NIWA 84727, TAN 0705-41. a) pleopod 1; b) telson; c) uropod 1; d) uropod 2; e) uropod 3. Scale bars: a; 200 µm; b–e; 100 µm.

Anterodistal group of long simple setae; merus, with angular posterodistal lobe short, narrow, setose; carpus strongly expanded, wider than propodus, posterodistal lobe subacute, distal margin oblique; propodus shorter than carpus, expanded distally, palm straight, crenulate; dactylus curved, just longer than palm. Pereopod 3 (Fig. 14c): coxa subequal to coxa 2, apex truncate; basis shorter than coxa, long plumose setae on posterior margin and close to anterior margin; merus weakly expanded anterodistally; carpus, length and breadth subequal to merus; propodus subrectangular, anterodistal and posterior margins setose; dactylus 1.4 × length of propodus. Pereopod 4 (Fig. 14d): coxa wider than long, anterior margin weakly convex, distal margin broadly rounded, anterodistal angle subrectangular, posterodistal lobe very strong, posterodistal angle rounded; basis shorter than coxa, anterior and posterior margins setose; merus weakly expanded anterodistally, setose; carpus 0.9 × merus, posterior margin convex, strongly setose, long setae anterodistally; propodus, anterodistal margin strongly setose, posterior margin setose; dactylus stout, straight, 1.8 × propodus. Pereopod 5 (Fig. 15a): coxa 0.9 × length of coxa 4, bilobed, posterior lobe expanded distally, distal margin straight, anterior lobe 0.6 × length of posterior lobe, rounded distally; basis shorter than coxa, few plumose setae on each margin; merus as long as basis, carpus 0.4 × length of merus; propodus slender, 0.7 × length of merus, shorter than the straight lanceolate dactylus; articles 2–6 variously setose. Pereopod 6 (Fig. 15c): coxa 0.7 × length of coxa 5, bilobed, posterior lobe subtriangular, anterior lobe 0.5 × length of posterior lobe; basis subrectangular, long plumose setae anterodistally; merus posterior margin convex; carpus subrectangular, 0.5 × length of merus; propodus 0.9 × length of merus; dactylus straight, lanceolate, as long as merus; articles 3–6 variously setose. Pereopod 7 (Fig. 15b): long; coxa wider than long, rounded posterodistally; basis, margins convex, posterodistal lobe nearly
as long as ischium; merus with groups of short setae on anterior and posterior margins [distal articles unknown].

**Pleon. Pleonites: 1–2 (Fig. 11a) with mid-dorsal, posteriorly directed carinate teeth; pleonite 3 lacking carina and tooth. Epimeron 1 and 3 broadly rounded posterodistally, epimeron 2 weakly angular. Pleopod 1 (Fig. 16a): peduncle and rami subequal.

**Urosome. Urosomite 1 (Fig. 11a): longest, with inconspicuous short boss close to the posterior margin; urosomites 2 and 3 subequal in length, lacking dorsal projections. Uropod 1 (Fig. 16c): peduncle elongate, lateral margin with dense row of short setae, inner margin with fewer and longer setae; inner ramus 0.7 × length of peduncle, both margins setose; outer ramus 0.9 × inner ramus, lateral margin setose. Uropod 2 (Fig. 16d): peduncle not tapering, both margins with short setae; inner ramus 0.9 × length of peduncle, both margins setose; outer ramus 0.9 × length of inner ramus, lateral margin setose. Uropod 3 (Fig. 16e): peduncle short, about as long as telson; rami subequal, 2.4 × length of peduncle, outer ramus with plumose setae on the lateral margin. Telson (Fig. 16b) tapered, notched 34%.

**Distribution.** Chatham Rise, east of New Zealand.

**Remarks.** The female specimen has the same antenna 1 morphology as the male: short peduncle articles and numerous flagellum articles. The proximal articles of the flagellum are shorter than wide.

**Oedicerina sp. indet.**

**Material examined.** 1 incomplete female; NHMUK 2014. 402, Discovery Stn 7845: north-eastern Atlantic, off the coast of Western Sahara: 23°50.5′N 17°05.9′W – 23°51.0′N 17°05.4′W, 24 March 1972, BN 2.4, 947–958 m.

**Remarks.** Only the head and pereonites 1–2 are present. Coxa 1–2 bear long setae along the distal margins. The animal appears similar to *O. ingolfi*, but as a result of incompleteness it is impossible to attribute it to any species.

**Discussion**

The three species described herein are morphologically very similar. Mouthparts and appendages show only minor and subtle differences and the species are best discriminated by habitus characters. Two of the species, *O. ingolfi* and *O. loerzae* sp. n. have mid-dorsal carinae on pleonites 1–2. *Oedicerina ingolfi* differs from *O. loerzae* sp. n. in having a small, slender, acute, upright tooth on pleonite 3 and a small pointed process on the posterior margin of urosomite 3 both of which are absent in *O. loerzae* sp. n. *Oedicerina vaderi* sp. n. has a small pointed process on the posterior margin of urosomite 3, as found in *O. ingolfi*, but pleonites 1–2 are evenly vaulted lacking any trace of a carina. Pleonite 3 of the holotype of *O. vaderi* sp. n. appears to be dorsally unarmed, but the paratypes have a small acute process (see female paratype, 6.3 mm in Fig. 10e). Coxa 5 of *O. ingolfi* is longer than wide, that of *O. vaderi* sp. n. is about as wide as long, and that of *O. loerzae* sp. n. much wider than long.

*Oedicerina sp. indet. was collected from the warm-temperate east Atlantic Ocean off Western Sahara. The unique specimen is incomplete, only the head and pereonites 1 and 2 are present, preventing a full identification.

Apart from the type species *O. ingolfi*, two other species had been described in the genus prior to this study: *Oedicerina megalopoda* Ledoyer, 1986, collected close to Mayotte, Mozambique Channel, western Indian Ocean (200–500 m) and *Oedicerina denticulata* Hendrycks & Conlan, 2003 from the northeast Pacific Ocean off California (4050 m). Knowledge of *O. megalopoda* is limited as the unique specimen is incomplete, but the massive rostrum of this species differs markedly from all other species of the genus. The palm of gnathopod 2 of *O. megalopoda* is straight, similar to that of *O. loerzae* sp. n., and thus different from the convex pattern seen in both North Atlantic species. In *Oedicerina denticulata* pleonite 1 is smooth and pleonites 2 and 3 have a postero marginal process. The process on pleonite 3 is directed posteriorly and is reminiscent of that seen in paratype material of *O. vaderi* sp. n. thus contrasting with the upright condition found in *O. ingolfi*. The posterior margin of urosomite 3 bears a small process (as do all species except for *O. loerzae* sp. n.) and urosomite 1 has a small upright process in the male but not the female, a character unique within the genus. Cox 5 appears to be longer than wide, as in *O. ingolfi*.

The mouthparts and appendages of all species of this genus are remarkably similar to each other. Examination of the extensive Norwegian Sea material which we attribute to *O. ingolfi* indicates that intraspecific variability is minimal, except for sexual dimorphism in antenna 1. In females of both Atlantic species peduncle articles of antenna 1 are longer and more slender than in males. Flagellum articles in females are uniformly much longer than wide and relatively few in number, whereas in males they are more numerous and proximally wider than long, forming an incipient calyptophore. This sexual dimorphism is not apparent in *O. loerzae* sp. n. where the structure of antenna 1 is very similar in males and the one ovigerous female paratype.

Because of minimal differences among appendages and mouthparts in *Oedicerina* species, differentiation within the genus relies significantly on patterns of ornamentation of pleonites and urosomites. As the posterior segments of the type material of *O. ingolfi* are missing, the question remains as to which of the two Atlantic species represents the species that Stephensen described. We allocate the material from the museums in Bergen and Lund studied herein to *O. ingolfi* on geographical grounds in that it was collected much closer to the type locality of that species, and on the morphological grounds of the dense fringe of setae on the distal margins of coxae 1 and 2 and the shape of the rostrum that our material shares with Stephensen’s original description.
Acknowledgments

We are very grateful to Wim Vader for inviting us to the highly successful identification workshop at Skibotn which provided the material that stimulated the present paper. We acknowledge with thanks Mr. Jon Anders Kongsrud (University of Bergen), Dr Lars Lunqvist (Museum of Zoology, Lund University) and Ms Sadie Mills (National Institute of Water and Atmosphere Research, Wellington, New Zealand) for the loan of material. We thank Ms Camilla Norrag, who drew the appendages of *O. ingolfi* and made the line drawings of *O. vaderi* sp. n.

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A new species of nocturnal gecko (*Paroedura*) from karstic limestone in northern Madagascar

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http://zoobank.org/CC6E4851-C6FD-4B47-973E-3E045390B579

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**Abstract**

*Paroedura hordiesi* sp. n. is described from Montagne des Français, a karstic limestone massif in the far north of Madagascar recently established as nature reserve. The new species has the nostril in contact with the rostral scale and shares many characters with *P. karstophila* and especially with *P. homalorhina* which are also restricted to karstic habitats. *Paroedura hordiesi* differs from *P. karstophila* by a smoother skin on dorsum and legs, by original and regenerated tails being both entirely smooth, by colouration, and by larger snout-vent length. Morphologically the new species is most similar to *P. homalorhina* from the Ankarana reserve from which it can be distinguished by shorter limbs and a less slender habitus. Published molecular data place the new species as close relative of *P. homalorhina* and another undescribed species from Nosy Hara Island, while newly determined data of the cox1 gene for *P. karstophila* confirm the distinctness of the new species from this taxon. Integrating the information from published and novel molecular data, the new species differs from all nominal *Paroedura* (except *P. vahiny* for which no molecular data are available to date) by strong genetic divergences. *P. hordiesi* might be another microendemic species of the Montagne des Français region. We suggest its IUCN Red List classification as “Critically Endangered” on the basis that it has an extent of occurrence of at most 50 km², it is known from a single location, and there is a continuing decline in the extent and quality of its habitat.

**Key Words**

Squamata
Gekkonidae
*Paroedura*
new species
Madagascar
Montagne des Français
conservation

**Introduction**

The far north of Madagascar comprises a mosaic of heterogeneous landscapes ranging from rainforests on volcanic basement to deciduous dry forests in karstic massifs and littoral habitats on sandy ground (e. g. Lavranos et al. 2001, Vences et al. 2009, Crottini et al. 2012). The geological and climatic diversity of this area is reflected by a high species diversity and a high degree of microendemism (e. g. Andreone 2004, Wilmé et al. 2006, Ranaivoarisoa et al. 2013). Endemism is usually restricted to the species level but one monotypic family of blindnakes (Xenotyphlopidae) is also endemic to this area (Wegener et al. 2013). Many new species of amphibians and especially reptiles have been discovered and described from far northern Madagascar in recent years, both from humid rainforests of the Montagne d’Ambre massif (e. g. Raxworthy and Nussbaum 2006, Glaw et al. 2007, 2010, Köhler et al. 2008, D’Cruze et al. 2010, Ratsoavina et al. 2011) and from the region around the dry karst forest formation of...
Montagne des François and adjacent littoral habitats (Ramanamanjato et al. 1999, Raselimanana et al. 2000, Glaw et al. 2001, 2005, 2012, 2013, Köhler et al. 2010a, 2010b, Miralles et al. 2011). These major habitat types are separated from each other by rather steep ecotones in northern Madagascar and thus in part constitute “habitat islands” for several species, possibly allowing allopatric speciation. Several taxa including dwarf frogs (Stumpffia), dwarf chameleons (Brookesia), burrowing skinks (Paracontias), leaf-tail geckos (Uroplatus), and the nocturnal geckos of the genus Paroedura have undergone remarkable diversification in northern Madagascar (Jackman et al. 2008, Köhler et al. 2010a, 2010b, Ratsaoavina et al. 2011).

The genus Paroedura is widely distributed throughout Madagascar’s biomes, including eastern rainforest, western dry forest, extremely arid thornbush savanna and high mountain habitats (Angel 1942, Guibé 1956, Dixon and Kroll 1974, Rösler and Krüger 1998, Nussbaum and Raxworthy 2000). Five of the 15 described species from Madagascar occur in the far north, and two additional species from the Comoro islands have close relationships to the northern P. lohatsara and P. stumpffi suggesting that they originated by two colonization events from the northern species (Hawlitschek and Glaw 2013). Recent surveys indicate that the karstic limestone massifs in this region still harbour further undescribed reptile species (e.g. Jackman et al. 2008, Recknagel et al. 2013). Some of them might be microendemic and threatened by substantial habitat destruction. In the following we describe a new Paroedura species from Montagne des Français to contribute to the taxonomic inventory of this massif, and to highlight the threats affecting this microendemic species and other biota in the region.

Methods

Specimens were anesthetized by injection with chlorobutanol, fixed with 96% ethanol and stored in 70% ethanol. Terminology and abbreviations of characters partly follow Nussbaum and Raxworthy (2000). Abbreviations used: MNHN = Muséum national d’Histoire naturelle, Paris; UADBA = Université d’Antananarivo, Département de Biologie Animale; ZSM = Zoologische Staatssammlung, München; SVL = snout-vent length. All measurements were taken with a caliper to the nearest 0.1 mm.

To obtain molecular comparisons of the new species with previously unstudied nominal Paroedura species, we sequenced a fragment of the mitochondrial gene for cytochrome oxidase subunit 1 (cox1) with primers and protocols defined by Nagy et al. (2012) for several Paroedura samples from Tsingy de Namoroka (corresponding voucher specimens to be catalogued in MNHN). These samples have become available through a recent herpetological survey by one of us (II) and include topotypical P. karstophila, previously unstudied from a molecular perspective. The resulting sequences were combined with those of Nagy et al. (2012) and Koubová et al. (2014) in MEGA version 5 (Tamura et al. 2011) to yield an alignment of 664 bp. We performed phylogenetic inference in MEGA under the Maximum Likelihood optimality criterion, with a general time-reversible substitution model with gamma-distributed rates and invariant sites, NNI branch swapping, and assessing robustness of nodes with 500 bootstrap replicates. Newly obtained sequences were submitted to GenBank (accession numbers: KM978078–KM978080).

Results

Molecular differentiation in the genus Paroedura

The multigene phylogenies of Jackman et al. (2008) and Hawlitschek and Glaw (2013) as well as the combined cox1 sequences of Nagy et al. (2012), Koubová et al. (2014) and those newly obtained (Fig. 1) provide evidence of strong genetic divergence of P. hordiesi to all other described Paroedura species except P. vahiny for which DNA sequence data is not yet available.

The cox1 data place the new species sister to an undescribed candidate species from Nosy Hara (79% bootstrap support), and this clade forms part of a more inclusive clade with P. oviceps and the undescribed Ankarafantsika species (bootstrap support 69%); no cox1 sequences were available for P. homalorhina from Ankarana which in a previous study was the sister taxon of P. hordiesi, see Jackman et al. (2008). All studied species and candidate species of Paroedura included in the cox1 data set were differentiated by very high pairwise uncorrected distances. P. hordiesi differed from its Nosy Hara sister lineage by a p-distance of 16.3–16.5%, and from all other Paroedura including the relatively distantly related P. karstophila by >20%. Two samples sequenced from the Tsingy de Namoroka, the type locality of P. karstophila, were sister to each other but showed a substantial divergence of 15.9% cox1 p-distance, suggesting that possibly two cryptic species may be hiding under the name P. karstophila in this karstic massif. The sample from Ankarafantsika (P. sp. Ankarafantsika in Fig. 1), identified in Jackman et al. (2008) as P. karstophila, in fact belongs to yet another, undescribed species according to our subsequent comparisons and is only distantly related to P. karstophila (Fig. 1). The DNA barcoding voucher sequence for the karyotype of P. karstophila (Koubová et al. 2014) is identical to our sequences of P. hordiesi, suggesting that the karyotype description in this paper refers to our new species rather than to P. karstophila.

Description of a new species

Paroedura hordiesi sp. n.

http://zoobank.org/8F5AA095-C68A-43E6-BCF8-4A02815A693F Figs 2–4, Table 1

Remarks. This species has been treated or figured under the name Paroedura homalorhinus (Henkel and Schmidt...
Figure 1. Maximum Likelihood tree inferred from 664 bp of the mitochondrial cytochrome oxidase subunit 1 (cox1) gene, showing the differentiation between *Paroedura* species. Note that this single-gene tree is not suitable to reconstruct the basal relationships of these geckos but is rather shown to document the large genetic divergences among all of them, and of *P. hordiesi* to its relatives.

Numbers behind species names are sample numbers as given in Nagy et al. (2012) and Koubouvá et al. (2014), as well as GenBank accession numbers of the respective sequences.
on 18–23 February 2004 by F. Glaw, M. Puente and R. D. Randrianainina; UADBA-R 70183 (FGZC 1109), adult male with everted hemipenes, UADBA-R 70185 (FGZC 1112), male, UADBA-R 70184 (FGZC 1114), adult female, ZSM 2106/2007 (FGZC 1099), juvenile, ZSM 2107/2007 (FGZC 1100), juvenile, ZSM 2113/2007 (FGZC 1115), adult female, all six collected around the remains of the French Fort (12°19'33''S, 49°20'17''E), collected on 27 February 2007 by P. Bora, H. Enting, F. Glaw, A. Knoll and J. Köhler; UADBA-R 70281 (FGZC 1659), sex unknown, ZSM 1530/2008 (FGZC 1660), adult female, both collected in the cave between Anda-vakoera and remains of French Fort, on 16 February 2008 by M. Franzen, F. Glaw, J. Köhler and Z. T. Nagy.

**Diagnosis.** *Paroedura hordiesi* sp. n. is a medium-sized species (SVL up to 58 mm, tail length up to 53 mm), having moderately prominent dorsal tubercles disposed into moderately distinct, and generally regular longitudinal rows and an original tail with no spines.

The new species can be easily attributed to the genus *Paroedura* based on its nested phylogenetic position within the genus (Jackman et al. 2008) and its morphological similarity to other *Paroedura* species, especially concerning the ventral structure of their fingers and toes which comprise a pair of squarish terminal adhesive pads. Among Malagasy geckos this terminal toe structure is only found in *Paroedura* and the related genus *Ebenavia* (Glaw & Vences, 2007). The latter genus can be easily distinguished from *Paroedura* by its much narrower and strongly pointed head, its elongated body, and smaller size.

**Comparisons.** The new species can be distinguished from the 17 other currently recognized *Paroedura* species (including the three available junior synonyms in the genus) as follows: *From P. androyensis, P. bastardi, P. ibityensis, P. lohatara, P. maingoka, P. picta, and P. vaziny* by having the nostril in contact with the rostral scale; from *P. gracilis* by absence of a raised vertebral ridge on the body and shorter forelimbs which are not extending forward beyond tip of snout; from *P. masobe* by much smaller size (SVL up to 58 mm versus 107 mm), much smaller eyes with a pigmented iris (versus black iris) and absence of a dorsal row of paired spines on the tail; from the two Comoroan species *P. sanctijohannis* and *P. stellata* by slightly smaller size (SVL up to 58 mm versus 68 mm and 62 mm, respectively) and absence of whorls with distinct spiny tubercles of the original tail; from the syntopically distributed *P. stumpffi* by smaller size (SVL up to 58 mm versus 70 mm) and absence of whorls with distinct spiny tubercles of the original tail; from *P. tanjaka* by much smaller size (SVL up to 58 mm versus 102 mm) and absence of whorls with distinct spiny tubercles of the original tail; from *P. vazimba* by larger size (SVL up to 58 mm versus 49 mm) and absence of whorls with distinct spines of the original tail; from *P. oviceps* from its type locality Nosy Be by smaller size (SVL up to 58 mm versus 69 mm) and rather regularly arranged tubercle rows on the back (versus rather irregular rows of tubercles); from *P. karstophila* by the absence of whorls with distinct spiny tubercles of the original tail (and by a smoother regenerated tail, see Nussbaum and Raxworthy 2000) and by
Table 1. Morphometric and meristic variation of several type specimens of Paroedura hordiesi from the type locality Montagne des Français. Abbreviations for measurements and counts (see Materials and Methods for other abbreviations): ZSM = Zoologische Staatssammlung München; SVL = snout-vent length; TL = tail length; HL = maximum head length (from tip of snout to posterior margin of ear); HW = maximum head width, at widest point; HH = maximum head height; AGL = axilla-groin distance; ED = maximum eye diameter; EO = maximum ear opening diameter; FOL = forelimb length, from axilla to tip of longest finger; HL = hindlimb length, from groin to tip of longest toe; SPL = number of supralimb scales; IFL = number of infralabial scales; NAS = number of nasals in direction from rostral to labial including nasorostrals, supranasals, postnasals; IN = number of internasals; IO = number of interorbitals; PM = number of postmentals; SLM4 = number of subdigital lamellae on fourth digit of manus; SLP4 = number of subdigital lamellae on fourth toe of pes; PCT = number of postcloacal tubercles; TLT = number of tubercle rows on tail. Counts are listed left-right. All measurements in Tables 1 and 2 were taken by H. Rösler with a caliper to the nearest 0.1 mm. HT = holotype, PT = paratype, F = female, M = male, SA = subadult.

| ZSM     | 352/2004 | 353/2004 | 340/2004 | 350/2004 | 339/2004 | 341/2004 | 338/2004 | 337/2004 | 342/2004 | 343/2004 |
|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Type status | PT | PT | PT | PT | PT | PT | PT | PT | PT | PT |
| Sex | F | F | F | F | F | F | F | SA | F | SA |
| SVL (mm) | 56.0 | 55.0 | 56.0 | 54.5 | 58.0 | 57.3 | 53.0 | 43.5 | 43.0 | 52.6 |
| TL | 48.5 | 16.7 | 17.0 | 17.0 | 16.4 | 18.7 | 18.0 | 14.9 | 14.8 | 16.3 |
| HL | 12.8 | 12.1 | 12.3 | 10.8 | 12.5 | 12.4 | 9.6 | 9.1 | 10.9 | 11.2 |
| HH | 8.0 | 7.1 | 7.2 | 6.3 | 7.4 | 6.6 | 5.8 | 5.1 | 6.4 | 5.7 |
| AGL | 25.0 | 22.0 | 23.0 | 22.8 | 23.0 | 23.7 | 17.0 | 18.1 | 21.7 | 21.0 |
| ED | 3.4 | 3.4 | 3.5 | 3.3 | 4.6 | 4.7 | 4.1 | 4.4 | 3.5 | 4.5 |
| EO | 2.2 | 1.9 | 1.7 | 1.9 | 1.8 | 1.8 | 1.5 | 1.5 | 1.7 | 1.7 |
| FOL | 20.0 | 19.8 | 20.2 | 20.0 | 20.6 | 21.2 | 16.2 | 17.4 | 18.6 | 18.7 |
| HIL | 27.0 | 25.1 | 28.8 | 28.7 | 28.6 | 30.0 | 24.5 | 24.4 | 27.5 | 26.4 |
| SVL:HIL | 2.07 | 2.19 | 1.94 | 1.90 | 2.03 | 1.91 | 1.78 | 1.76 | 2.07 | 1.96 |
| SPL | 15.15 | 15.15 | 15.15 | 14.15 | 16.15 | 15.15 | 16.15 | 15.15 | 16.15 | 15.15 |
| IFL | 13.13 | 13.13 | 12.13 | 13.13 | 13.13 | 13.13 | 13.13 | 13.13 | 13.13 | 13.13 |
| NAS | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 | 5–5 |
| IN | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| IO | 6 | 6 | 6 | 7 | 7 | 7 | 8 | 6 | 7 | 6 |
| PM | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| SLM4 | 13–13 | 14–14 | 14–16 | 15 | 17–15 | 16–16 | 15–15 | 15–15 | 15–15 | 15–15 |
| SLP4 | 16–18 | 17–18 | 19–19 | 19–18 | 20–20 | 18–18 | 18–17 | 17–18 | 19–19 | 19–19 |
| PCT | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 | 1–1 |
| TLT | 7 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |

colouration (see Fig. 2 versus Fig. 5); and from its close relative P. homalorhina (Jackman et al. 2008) by shorter limbs (finger tips reach the anterior margin of eye versus snout tip when forelimbs are addressed along the body), slightly smaller size (SVL up to 58 mm versus 65 mm, see Table 2), distinct and generally regularly arranged tubercles rows on the back (versus less distinct and less regular rows), and a more slender habitus (see Fig. 2 versus Fig. 5). In addition, Paroedura hordiesi can be easily distinguished from most other Paroedura species (P. androyensis, P. bastardii, P. gracilis, P. ibityensis, P. lohatsara, P. maingoka, P. masobe, P. oviceps, P. picta, P. sanctijohannis, P. stellata, P. stumpffi, P. tanjaka, P. vahiny and P. vazimba) by adult colouration in life (see colour photographs in Glaw and Vences 2007, Schönecker 2008, Hawlitschek and Glaw 2013) and from P. androyensis, P. bastardii, P. gracilis, P. homalorhina, P. ibityensis, P. lohatsara, P. maingoka, P. masobe, P. picta, P. sanctijohannis, P. stellata, P. stumpffi, P. tanjaka, and P. vazimba by juvenile colouration (Glaw and Vences 2007, Schönecker 2008, Hawlitschek and Glaw 2013, FG pers. obs.; juvenile colouration of the other species still unknown). Genetically, P. hordiesi can be distinguished from all other species in the genus by its molecular differentiation in mitochondrial and nuclear genes (Jackman et al. 2008, Nagy et al. 2012, Hawlitschek and Glaw 2013, Fig. 1) except for P. vahiny for which DNA sequences are not yet available.

Description of the holotype. SVL 52.6 mm, further measurements and counts are given in Table 1. Holotype in good condition, with complete but broken original tail and everted hemipenes. Head distinctly wider than neck and wider than body. Snout angled downward to tip, slight depression between poorly developed canthal ridges. Ear opening is a vertical slit. Original tail slightly shorter than snout-vent length, nearly round in cross section in its proximal part, laterally compressed in its distal half, with sharply pointed tip; ventral pygal section with pair of postcloacal sacs. Digits moderately expanded at tips. Rostral scale rectangular, much wider than tall and wider than mental. Nostril in contact with
rostral, first supralabial, and five further scales. First supralabial largest, labials smooth. Snout and interorbital scales juxtaposed, some raised, some scales in front of orbits tuberculate, as are some larger lateral occipital scales. Dorsolateral neck and body scales very heterogeneous with about eight partly poorly recognizable longitudinal rows at midbody of enlarged tubercles; enlarged tubercles separated partly by small flat scales and smaller tubercles. Dorsal scales of forelimbs mostly flat. Dorsal scales of hindlimbs large and tuberculate, much smaller above knee. Ventral scales of forelimbs slightly smaller than surrounding ventral scales of the body. Dorsal pygal scales like dorsal body scales; lateroventral pygals tuberculate. Tail scales flat, tail segments without any transverse row of spiny tubercles. Mental triangular, bordered posteriorly by a pair of elongate, irregular hexagonal postmentals. Postmentals contact mental, first and second infralabial, one enlarged lateral gular, one smaller posterolateral gular, and one larger central gular. First three infralabials not significantly larger than others. Gulars small, granular. Ventrals of chest and abdomen flat. Proximal subdigitals in rows of 2–3. Pair of squarish, terminal pads. Claws curving downwards between terminal pads of digits.

Colour after 10 years in alcohol (Fig 3): head dorsally beige with almost no recognizable pattern except a whitish dorsolateral spot above each ear opening and a well-defined blackish area above each eye which represents, however, no pigmentation of the skin, but is due to the blackish eyeball shining through the skin. Dorsum greyish with four poorly defined beige spots on the back which are the remains of dorsal crossbands: one distinct spot between the forelimbs, two poorly recognizable spots on the back between forelimbs and hindlimbs, and a fourth poorly recognizable spot between the hindlimbs. Dorsal surfaces of forelimbs and hindlimbs marbled with beige and grey. Flanks with similar colour as dorsum, but without any recognizable traces of light crossbands. Tail dorsally with whitish-grey and brown alternating transverse bands which are poorly delimited in the distal portion of the tail. Throat, chest, venter, ventral parts of forelimbs and hindlimbs and ventral side of tail whitish. Iris dark grey, pupil white. Colour of holotype in life unknown (no colour photographs available).
Figure 5. Paroedura karstophila from its type locality Tsingy de Namoroka (A), observed in the night of 10 September 2012 around 22:50 (not collected), and P. homalorhina from its type locality Ankarana (B).
Variation. Morphometric and meristic variation of ten specimens of the type series is summarized in Table 1, but there is also a remarkable individual variation in dorsal colouration and pattern (Fig. 2). Paratype ZSM 2106/2007, the smallest known juvenile (SVL 28.1 mm, tail length 25.2 mm), shows a moderately distinct juvenile colouration both in life (Fig. 2) and in preservative consisting of four light crossbands on the back between the insertion of forelimbs and the insertion of hindlimbs. A similarly distinct banding on body and original tail is still visible in the larger juvenile paratype ZSM 2107/2007 (Fig. 2, SVL 35.2 mm, tail length 34.7 mm). Although the juvenile colouration is distinct compared to most adults, it is less colourful and less contrasting in comparison with many other Paroedura species. The adult paratypes usually have a less distinct dorsal colour pattern than the juveniles, ranging from poorly recognizable (e.g. ZSM 532/2000) to distinct and well delimited (e.g. ZSM 531/2000) after 14 years in alcohol. Additional photographs of living individuals are provided in Henkel and Schmidt (1995), Glaw and Vences (2007), and Schönecker (2008). Most of the adult type specimens have a regenerated tail (without distinct transversal banding of dark and white), indicating a high pressure by predation or intraspecific aggression.

Hemipenis. The following description is based on the everted right organ of ZSM 343/2004 (Fig. 4). Hemipenis medium-sized (total length 6.4 mm, width at apex 4.5 mm), apex divided in two lobes. Sulcus spermaticus forms an S-shaped, narrow and deep groove, bordered by moderately distinct lips reaching the apex. On the apex, the sulcus is becoming broader and divides in two branches. A horn-like apical cone is present in the center of each lobe. Calyces on the apex are only present at the border of the lobes. The hemipenes of the holotype (ZSM 342/2004) are virtually identical in every respect.

Habitat and habits. Paroedura hordiesi was observed multiple times at night in karstic dry forest in the rainy season, mainly climbing on karstic rocks and the ruins of an old fort. It was found in close syntopy with P. lohatsara in the karstic limestone areas, whereas P. stumpffi was only encountered on the slope between the massif and the sea, in areas without karstic formations.

Etymology. The specific name is dedicated to Freddy Hordies, in recognition of his support for biodiversity research and conservation through the BIOPAT initiative.

Distribution and conservation status. Paroedura hordiesi is reliably known only from the recently established nature reserve of Montagne des Français. The species possibly also occurs at Ampombofofo, ca. 25 km north of this massif (Megson et al. 2009: ZSM 1531/2008), but the identity of this population needs further study as molecular data are not available thus far. Surveys in other limestone areas of northern Madagascar revealed a superficially similar Paroedura at Nosy Hara (Metcalf et al. 2007) which is, however, strongly differentiated by colouration (see photo in Glaw and Vences 2007) and mitochondrial DNA sequences (Fig. 1 and Nagy et al. 2012), and is therefore considered a further undescribed candi-

Table 2. Morphometric and meristic variation of Paroedura homalorhina from the type locality Ankaranana. See Table 1 for abbreviations and further details.

| ZSM   | 345/2004 | 347/2004 | 348/2004 | 346/2004 | 349/2004 | 344/2004 | 768/2003 | 781/2003 |
|-------|----------|----------|----------|----------|----------|----------|----------|----------|
| Sex   | M        | F        | F        | M        | F        | F        | F        | M        |
| SVL   | 64.8     | 58.6     | 58.8     | 61.5     | 61.3     | 61.7     | 60.4     | 62.8     |
| TL    | 68.0     | 60.0     | 60.0     | 61.0     | 61.0     | 61.0     | 61.0     | 61.0     |
| HL    | 19.6     | 17.6     | 17.7     | 19.0     | 17.8     | 19.2     | 18.3     | 18.7     |
| HW    | 12.6     | 12.0     | 11.4     | 12.9     | 12.2     | 12.6     | 11.3     | 12.9     |
| HH    | 7.3      | 7.2      | 6.4      | 7.2      | 7.0      | 7.5      | 7.2      | 7.0      |
| AGL   | 26.8     | 24.0     | 23.0     | 27.8     | 28.0     | 27.1     | 26.4     | 24.4     |
| ED    | 3.8      | 3.5      | 4.3      | 4.3      | 3.8      | 4.0      | 4.7      | 4.4      |
| EO    | 1.6      | 1.7      | 1.5      | 1.6      | 1.8      | 1.6      | 1.7      | 1.6      |
| FOL   | 23.9     | 23.8     | 22.4     | 25.2     | 21.7     | 25.4     | 24.1     | 25.7     |
| HIL   | 36.4     | 36.6     | 33.0     | 39.2     | 35.3     | 33.0     | 34.5     | 35.8     |
| SVL:HL| 1.78     | 1.60     | 1.78     | 1.57     | 1.74     | 1.87     | 1.75     | 1.75     |
| SPL   | 15-14    | 16-15    | 16-15    | 15-15    | 14-14    | 15-15    | 15-14    | 15-16    |
| IFL   | 13-13    | 13-13    | 12-14    | 13-12    | 14-13    | 12-13    | 13-12    | 13-12    |
| NAS   | 5-5      | 5-5      | 5-5      | 5-5      | 5-6      | 6-6      | 6-6      | 5-5      |
| IN    | 1        | 1        | 1        | 1        | 1        | 1        | 1        | 1        |
| IO    | 6        | 6        | 6        | 6        | 6        | 6        | 6        | 6        |
| PM    | 2        | 2        | 2        | 2        | 2        | 2        | 2        | 2        |
| SLM4  | 16-16    | 15-16    | 16-16    | 16-16    | 17-17    | n.a.-17  | 16-16    | 15-14    |
| SLP4  | 18-19    | 18-19    | 18-17    | 20-20    | 19-20    | 19-19    | 19-19    | 19-18    |
| PCT   | 1-1      | 1-1      | 1-1      | 1-1      | 1-1      | 1-1      | 1-1      | 1-1      |
| TLT   | 3        | 5        | 2        | 2        | 2        | 2        | 2        | 2        |
date species. The Ankaran massif is known to harbour *P. homalorhina*, *P. karstophila*, and *P. stumpffi* (Nussbaum and Raxworthy 2000, Raselimanana 2008). Our own surveys in Ankaran revealed only *P. homalorhina* and *P. stumpffi* and we consider the occurrence of *P. hordiesi* unlikely in this massif. Rakotondravony (2006a) found *Paroedura* sp. at Binara in the Loky-Manambato region which potentially could refer to *P. hordiesi*, but the identity of this record remains to be studied. No unidentified *Paroedura* species was found at Analamera (Rakotondravony 2006b). Thus current evidence suggests that *P. hordiesi* is microendemic of Montagne des Français and perhaps the adjacent Ampombofo region.

For consistency with the IUCN Red List Assessment for *Paroedura lohatsara* (Raxworthy et al. 2011) and other potential microendemic species of the Montagne des Français region, we suggest a classification as “Critically Endangered” on the basis that *P. hordiesi* has an extent of occurrence of at most 50 km², it is known from a single location, and there is a continuing decline in the extent and quality of its habitat.

Discussion

With the description of *Paroedura hordiesi* we add a further, probably microendemic new species to the herpetofauna of Montagne des Français. Although this population is already known for approximately 20 years, several factors have hampered the clarification of its identity, including the variability in colouration and in distinctiveness of longitudinal rows of dorsal tubercles of *P. hordiesi*, *P. homalorhina* and *P. karstophila*, the existence of several undescribed species with similar key characters, the rarity of individuals with an original tail, and the absence of genetic data and colour photographs reliably referable to *P. karstophila*. This situation has led to substantial uncertainty about the correct name for the species from Montagne des Français, as is reflected by the different preliminary names that have been used for this species in the literature (see above). DNA sequences are now available from two *P. karstophila*-like specimens from the type locality Namoroka (Fig. 1). These strongly differ from each other, suggesting that *P. karstophila* is possibly a composite taxon including two cryptic species which occur in syntopy at Namoroka. Although the identity of *P. karstophila* remains to be further studied, we have little doubts that one of the two Namoroka lineages will turn out to correspond to this taxon.

Due to PCR failure with universal reptile primers (Nagy et al. 2012), no cox1 sequences are available for several species of *Paroedura*, including *P. homalorhina* which was sister to *P. hordiesi* in the study of Jackman et al. (2008; as *P. sp. n.*). On the other hand, the new species from Nosy Hara and *P. karstophila* have not yet been sequenced for the genes used by Jackman et al. (2008). This unfortunate lack of overlap of the two molecular data sets, to be remedied by future sequencing efforts, hampers our ability to determine whether *P. hordiesi* is more closely related to *P. homalorhina*, or to the undescribed species from Nosy Hara. It does not, however, compromise our taxonomic conclusion of *P. hordiesi* being a valid species differentiated from both these close relatives by a substantial genetic divergence and morphological characters.

*Paroedura hordiesi* is a typical example of a microendemic karst specialist of an isolated habitat island that apparently has lost its ability to survive outside its special habitat and thus has lost the genetic exchange with populations from neighbourd karstic massifs. Although the surrounding massifs are only separated by several kilometers of grassland or other non-karstic habitats, they are often populated by different species which might have evolved in isolation for millions of years. The cox1 data presented in Fig. 1 suggest that the undescribed *Paroedura* species from the island Nosy Hara is a close relative of *P. hordiesi*. The two species differ by a substantial genetic distance, and distinctly by their colouration (see photographs in Glaw and Vences 2007), although the distance between this island and Montagne des Français is just about 35 km. The distance between Montagne des Français and Ankaran is approximately 50 km, yet the latter massif is populated by *P. homalorhina*, the second close relative of *P. hordiesi* (see discussion in previous paragraph). Similar patterns of microendemism and comparable phylogenetic relationships have been found in dwarf chameleons of the genus *Brookesia* (Glaw et al. 2012), in a clade of dwarf frogs of the genus *Stumpfia* (Köhler et al. 2010a), and in lizards of the genus *Zonosaurus* (Raselimanana et al. 2009, Recknagel et al. 2013), suggesting that similar vicariant processes among karstic habitat islands might have affected these groups of organisms in the far north of Madagascar. The karstic massifs might have provided sufficient resources and protection from desiccation during periods of drier climate to allow the long-term survival of these populations, finally leading to completed speciation. The evolution and long-term survival of these microendemic species suggests rather stable conditions without catastrophic events over very long time periods. The apparent lack of gene flow among the karstic habitat islands also suggests a limited dispersal capacity of karst specialists across the non-karstic interspersed matrix.

Acknowledgements

We are very grateful to Parfait Bora, Hildegard Enting, Michael Franzen, Kathrin Glaw, Angelika Knoll, Steven Megson, Zoltan T. Nagy, Marta Puente, Roger-Daniel Randrianiaina, Angelin Razafimantsoa and the team of the Kings Lodge for their help in the field, to York Pareik for logistic support, and to two anonymous reviewers for their helpful comments on the manuscript. Special thanks go to Freddy Hordies for his dedication for biodiversity research and conservation and his patience. The research in Madagascar was made possible.
by a cooperation accord between the UADBA and the ZSM. Malagasy authorities are acknowledged for collection and export permits. The research of F. Glaw was supported by the “Deutsche Forschungsgemeinschaft” (grant no. GL 314/1) and the European Association of Zoos and Aquaria (EAZA). Fieldwork of I. Ineich was made possible with the help of Ewe Madagascar for logistical support (Marc Gansuana), La Colas and the Société Aquamas for providing boats, thanks to them. He also wants to thank Lucile Allorge and Thomas Haevermans (MNHN) for scientific organisation, MNHN Labex for providing founds, and the Parc naturel du Namoroka, its guides and the inhabitants of the village of Vilanandro for their useful help.

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Glaw, F. et al.: New Paroedura from Madagascar
The anuran fauna of a Volunteer Nature Reserve: the Tanoé-Ehy Swamp Forests, south-eastern Ivory Coast, West Africa

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Abstract

We report the results of an amphibian survey in the Tanoé-Ehy Swamp Forests, south-eastern Ivory Coast. During 26 days we recorded at least 33 frog species. These include a new record for the recently described Morerella cyanophthalma, which seems to be endemic to the south-eastern Ivorian forests. Some individuals of the genus Phrynobatrachus may represent the so far unknown males of P. intermedius, described from neighboring Ghana and only known from the type locality, or a species new to science. Based on the IUCN Red List more than one quarter of the recorded species are threatened. The study sites comprise an amphibian fauna mainly consisting of forest specialists that are endemic to the Upper Guinea forest zone. Although some regional endemics were lacking from our records, the Tanoé-Ehy Swamp Forests have great value for amphibian conservation in Ivory Coast. However, the presence of some invasive species is a clear hint to past and present forest alteration. We urge for the protection of these forests as they represent an important refuge for the forest fauna in the south-eastern Ivory Coast, a region where only few forests persisted until today.

Key Words
Amphibia
Anura
biodiversity
conservation
degradation
diversity
endemicity
hotspot
Upper Guinea

Introduction

Most West African countries have lost the majority of their natural forests. Ivory Coast has been experiencing a great loss of rainforest cover in the west (e.g. Chatelain et al. 1996), and this has been worsening during the political crisis in the first decade of the 21st century (Bible 2013, Hansen et al. 2013). This situation is also severe in other parts of this country, although forest loss in these other areas has received much less attention. The rainforests in south-eastern Ivory Coast are actually among the most highly threatened African forests (Norris et al. 2010, Mayaux et al. 2013). In particular they face logging, shifting agriculture and poaching (Lauginie 2007). Although, since 1926, the Ivorian State created protected areas throughout the country (Lauginie 2007), the eastern forested areas were neglected (Bakarr et al. 2004). To reinforce its conservation policy, the Ivorian state is now encouraging compensatory measures, i.e. the creation of volunteer nature reserves (VNR), in addition to protected areas. One of these VNRs is the Tanoé-Ehy Swamp Forests, which has been designated as a “Very High” priority area for primates. To further enhance protection it has been recommended to collect...
and update the scientific information for this forest area (Koné et al. 2008).

Recently it has been shown that the amphibian faunas of the western (western Ivory Coast and westwards) and eastern (eastern Ivory Coast to the Dahomey Gap in Benin and Togo) Upper Guinea forests are distinctly different (Penner et al. 2011). The discovery and descriptions of several new species from eastern Ivory Coast and western Ghana, further underline the faunistic uniqueness of this region (Assemian et al. 2006, Rödel et al. 2009a, 2009b, Rödel et al. 2012, Kouamé et al. 2014a).

So far the scientific knowledge of the Tanoé-Ehy Swamp Forests is fragmentary (Koné and Akpatou 2004, Ahon 2010, Zadou et al. 2011) and completely excludes amphibians. However, amphibians are important parts of tropical ecosystems and provide numerous ecosystem services (Mohneke and Rödel 2009, Hocking and Babbitt 2014). Furthermore representatives of this taxonomic group are known to react sensitively to habitat alteration. The composition of amphibian assemblages thus may reflect the degree of habitat degradation and destruction (for Ivorian examples compare e.g. Ernst and Rödel 2005, Ernst et al. 2006, Hillers et al. 2008). Hence, conservation recommendations could be based on the presence or absence of particular amphibian species and their continued monitoring.

The Tanoé-Ehy Swamp Forests have recently been the focus for exploitation by an agro-industrial company, making a comprehensive survey of its biological richness more pressing. We therefore participated in a survey of the Tanoé-Ehy Swamp Forests organized by the “Centre Suisse de Recherche Scientifique.” The amphibian data are presented in this paper.

**Material and methods**

The Tanoé-Ehy Swamp Forests (TESF; 5°05′–5°15′ N; 2°45′–2°53′ W) constitute 12,000 ha of remaining rainforest in the department of Tiapoum, south-eastern Ivory Coast. The mean annual temperature is 26 °C, the mean annual precipitation is about 2000 mm. A longer dry season lasts from December to March, and is followed by the period with highest precipitation in March to July. A minor rainy season extends from October to November (Eldin 1971). The River Tanoé crosses the southern and eastern parts of the TESF while the western part of these forests is marked by the Ehy lagoon (Fig. 1). The TESF mainly consist of moist, partly primary forests on predominantly sandy soil with vegetation typical for south-eastern Ivory Coast (Béligné 1994).

The survey was carried out in the northern part of TESF from 17 June to 29 July 2010 (long rainy season) and from 19 September (minor dry season) to 5 October 2010 (minor rainy season). We searched for frogs along eight sites during day and night. Searching techniques included visual
search for frogs, the investigation of potential hiding places or very specific habitats (e.g. exceptional breeding sites such as water-filled tree holes), and the acoustic monitoring of frog calls (see Heyer et al. 1994, Zimmerman 1994, Rödel and Ernst 2004). All available habitats were examined by two people. We searched for frogs on 26 days, each day for seven hours (07:00–11:00 h & 19:00–22:00 h GMT). The sampling effort therefore was always 14 person-hours per day and thus comparable throughout the survey. A GPS receiver (Etrex venture HC Garmin) was used to record geographic positions. Coordinates and short site descriptions are given in the Appendix 1. As our sampling design provides only qualitative and semi-quantitative data we calculated the estimated species richness, and thus the sampling efficiency, with the Chao 2 and Jack-knife 1 estimators (software: EstimateS, Colwell 2006). These estimators are incidence based, calculating using the presence/absence data of the daily species lists (26 days of survey work) for 33 species. To avoid order effects we accomplished 500 random runs of the daily species lists.

Encountered individuals were usually determined to species level and the nomenclature used herein follows Frost (2014). *Arthroleptis* species in the Upper Guinean forests are currently difficult to identify because of overlapping intraspecific and interspecific variation in morphology (Rödel and Bangoura 2004). Judging from their advertisement calls our *Arthroleptis* records comprise more than one species, but are treated herein as one taxon *Arthroleptis* spp. Snout-vent-lengths (SVL) of the living frogs were taken with a dial caliper (accuracy ± 0.5 mm). Voucher specimens of all frog species were euthanized in a chlorobutanol solution and preserved in 70% ethanol. Voucher specimens are deposited at the “Laboratoire de Zoologie et de Biologie Animale” at the Félix Houphouët Boigny University, Abidjan. Some specimens of particular interest (see below) have been deposited in the Museum für Naturkunde, Berlin (ZMB).

**Results**

*Species richness and community composition.* Overall, we recorded 33 anuran species in nine families and 13 genera. A total species list with sites records (compare Appendix 1), known habitat preferences, distribution and IUCN Red List Category (IUCN 2013) is given in Table 1. The comparison of the species accumulation curve to the two incidence-based species richness estimators revealed that more amphibian species could probably be encountered within the TESF (Fig. 2). The Jack-knife 1 estimator calculated 39 (sd: ± 2.3) species for the area, the Chao 2 estimator estimated 40 (sd: ± 8.6) species. We hence probably recorded about 77.5–79.5% of the local species pool. Voucher specimens are deposited at the “Laboratoire de Zoologie et de Biologie Animale” at the Félix Houphouët Boigny University, Abidjan. Some specimens of particular interest (see below) have been deposited in the Museum für Naturkunde, Berlin (ZMB).

![Figure 2. Species accumulation curve (Sobs) and estimated amphibian species richness (Chao 2 and Jack-knife 1). Mean and positive values of standard deviation shown)](image-url)

Figure 2. Species accumulation curve (Sobs) and estimated amphibian species richness (Chao 2 and Jack-knife 1; mean and positive values of standard deviation shown) of the Tanoé-Ehy Swamp Forests, south-eastern Ivory Coast.

We recorded the highest species numbers at KA (21 species), followed by RE (20), MN, EB, YA (each 10). Species richness was lowest at MK (8), KW (7) and PN (5). While the site RE consisted of a swampy forest with *Raphia* palms and partly open canopy, EB, KA and KW comprised dense vegetation characterized by large canopy gaps and thick shrubby undergrowth along a river. At KA we recorded creeks, numerous puddles and ponds, and more leaf-litter compared to other sites. The site PN, which was relatively dry, mainly consisted of a more uniform forest with dense canopy. The four other sites (MK, MN and YA) were partly degraded and comprised also rice fields, small farms or oil palm plantations.

The majority of the encountered species are closely associated with forest habitats (13 species, 39.4%). Eight species (24.2%) predominantly occur in forest, but tolerate degraded habitats such as farmbush (secondary growth or degraded forest) or even savanna (Table 1). Twelve species (36.4%) usually prefer savanna and farmbush habitats and normally do not occur in pristine forests. At most survey sites, the amphibian assemblages were dominated by forest species (Table 1). Nevertheless, we observed species with preferences for farmbush and savanna habitats at all sites, suggesting that the area has already suffered from habitat degradation.

Most recorded species (54.5%) do not occur outside West Africa (defined as the area west of the Cross River in Nigeria), and are often restricted to smaller parts of West Africa. More than one quarter (27.3%) of all recorded species only occur in the Upper Guinea forest zone (forests west of the Dahomey Gap), while three records (*Morerella cyanophthalma*, *Phrynobatrachus ghanensis* and *P. cf. intermedius*) are potentially endemic to the forests in south-eastern Ivory Coast and adjacent Ghana.

According to the IUCN Red List, almost a quarter (24.2%) of all recorded species are threatened or near threatened: four species are Near Threatened (*Afrixalus nigeriensis*, *Leptopelis occidentalis*, *Phrynobatrachus allenii* and *P. liberiensis*), two are Vulnerable (*Kassina arboricola* and *Morerella cyanophthalma*), and two *Hylarana occidentalis* and *Phrynobatrachus ghanensis*, are Endangered (IUCN 2013; Table 1). If the *Phrynobatrachus* sp. males are *P. intermedius* (see below), the list would also comprise a Critically Endangered species.

**Notes on selected species.** We only comment on four species of particular interest. *Afrixalus fulvovittatus fulvovittatus* (Cope, 1861) was described from Liberia and is mainly distributed in the
western Upper Guinea forest zone. Our present records (Fig. 3) confirm the species’ presence in south-eastern Ivory Coast (Pickersgill 2007).

*Morerella cyanophthalma* Rödel, Assemian, Kouamé, Tohé & Perret, 2009 (Fig. 4) is a recently erected monotypic genus (Rödel et al. 2009a), known so far only from the type locality, Banco National Park in Abidjan, and Azagny National Park, west of Abidjan (the latter locality was recently confirmed by N.G. Kouamé et al., unpubl. data). In TESF, a few of these frogs could be found in the swampy parts of the forest, dominated by *Raphia* palms. While two males and two females were found in disturbed forest parts next to a road, another female was seen in a forested part at the edge of a rice field. One male was seen

### Table 1. Amphibian species recorded in the Tanoé-Ehy Swamp Forests with recorded sites (see Appendix), general habitat preferences, distribution and IUCN Red List category (IUCN 2013).

| Family / Species | Site | Habitat | Distribution | IUCN Red List |
|------------------|------|---------|--------------|---------------|
| **Arthroleptidae** |      |         | S | FB | F | A | WA | UG | E |
| *Arthroleptis* spp.* | RE | X | X | X | LC |
| *Leptopelis occidentalis* | KA | X | X | NT |
| *L. spinusnoctis* | EB, KA, KW, YA | X | X | X | LC |

| **Bufonidae** |      |         | S | FB | F | A | WA | UG | E |
| *Amietophrynus maculatus* | KA, RE | X | X | X | LC |
| *A. regularis* | EB, MN, RE | X | X | X | LC |

| **Dicroglossidae** |      |         | S | FB | F | A | WA | UG | E |
| *Hoplobatrachus occipitalis* | KA, RE, YA | X | X | X | LC |

| **Hyperoliidae** |      |         | S | FB | F | A | WA | UG | E |
| *Afrixalus dorsalis* | EB, KA, MN, RE, YA | X | X | X | LC |
| *A. fulvovittatus fulvovittatus* | RE | X | X | X | LC |
| *A. nigeriensis* | RE | X | X | NT |
| *Hyperolius concolor concolor* | EB, KA, KW, MK, MN, RE, YA | X | X | X | LC |

| **Hyperolius guttulatus** | KW, MK, MN, RE | X | X | LC |
| **Hyperolius picturatus** | KW, RE | X | X | X | LC |
| **Hyperolius sylvaticus** | KA | X | X | LC |
| **Kassina arboricola** | KA | X | X | VU |

| **Morerella cyanophthalma** | MK, RE | X | X | VU |

| **Phrynobatrachidae** |      |         | S | FB | F | A | WA | UG | E |
| *Phrynobatrachus alleni* | PN | X | X | NT |
| *P. calcaratus* | KA, MK | X | X | X | LC |
| *P. ghanensis* | KA | X | X | EN |
| *P. gutturosus* | EB, KA, KW | X | X | X | LC |
| *P. sp. (cf. intermedius)* | RE | X | X | CR |
| *P. latifrons* | EB, KA, KW, MK, MN, RE, YA | X | X | X | LC |
| *P. liberiensis* | RE | X | X | NT |
| *P. plicatus* | KA | X | X | LC |

| **Pipidae** |      |         | S | FB | F | A | WA | UG | E |
| *Silurana tropicalis* | RE | X | X | X | LC |

| **Ptychadenidae** |      |         | S | FB | F | A | WA | UG | E |
| *Ptychadena bibroni* | EB, MN, YA | X | X | X | LC |
| *P. longirostris* | EB, KA, RE | X | X | X | LC |
| *P. mascareniensis* | KA, MK, PN | X | X | X | LC |
| *P. oxyrhynchus* | KA | X | X | LC |
| *P. pumilio* | KA, MK, MN, RE, YA | X | X | X | LC |

| **Pyxicephalidae** |      |         | S | FB | F | A | WA | UG | E |
| *Aubria subsigillata* | KA, RE, YA | X | X | LC |

| **Ranidae** |      |         | S | FB | F | A | WA | UG | E |
| *Hyla arenicolor* | KA, MN, PN, RE | X | X | X | LC |
| *H. occidentalis* | KA, MN, PN, RE | X | X | EN |
during the day on the ground. The four other individuals were captured at night perched on *Raphia* palms.

In sun light the frogs have the horizontal oval pupil shape typical for the genus (Fig. 4a); males possess a medium-sized medioposterior gular gland without dilatable skin beneath and around the gland (Fig. 4b); males bear small spines on the back and extremities, being most pronounced on the external side of toe V; and the sexes exhibit a pronounced sexual color dimorphism (Fig. 4). In contrast to frogs from the type locality, the males from TESF reached 23.5–26.6 mm SVL (largest male: ZMB 80869), the females 31.0–32.0 SVL, thus showing a more pronounced sexual size dimorphism and smaller sizes (Rödel et al. 2009a). However, these differences could be also due to a much smaller sample size at TESF, compared to the series from Banco National Park. The dorsal spines in ZMB 80869 are less pronounced than in the type series, with spines on the back, femur, tibia and tarsus being difficult to see; only those spines on the external side of toe V are immediately visible. The sexual dichromatism was similar to the type series. However, in contrast to females from Banco forest (iris colored blue), some females from TESF had a reddish brown iris (Fig. 4c).

We believe that the frogs presented herein represent the easternmost known record of *Morerella cyanophthalma*, approximately 177 km from the type locality. However, the small morphological differences mentioned above urge for a molecular and acoustic examination of the newly discovered population.

**Figure 3.** *Africalus fulvovittatus fulvovittatus* from the Tanoé-Ehy Swamp forests.

*Hylarana occidentalis* (Perret, 1960) was so far known from primary or nearly primary rainforests in Ghana (Perret 1977, 1983, Rödel et al. 2005), western Ivory Coast (Rödel and Branch 2002), south-eastern Guinea (Böhme 1994, Rödel et al. 2004), Liberia (Hillers and Rödel 2007) and Sierra Leone (A. Hillers and M.-O. Rödel, unpubl. data). This is only the second record for south-eastern Ivory Coast (compare Kouamé et al. 2014b).

*Phrynobatrachus* sp. We found adult *Phrynobatrachus* males (ZMB 80870-80871) which are not assignable unambiguously to a described species (Fig. 5). Superficially they resemble most either *P. liberiensis* Barbour & Love-ridge, 1927 or *P. intermedius* Rödel, Boateng, Penner & Hillers, 2009. With both they share, amongst other characters, the general body shape, as well as the shape of a pair of feebly developed scapular ridges. From *P. liberiensis* males, they differ by a darker patterning on the breast, more numerous and more distinct spines on the back (in particular on the posterior-most part of the back), more distinct and denser spines on the black throat, more dark dots on the belly and a blackish facial mask. With *P. intermedius* they share the black facial mask. The latter species is only known from two females and a juvenile from the primary forest of the Ankasa Reserve in south-western Ghana (Rödel et al. 2009b). Males of this species have not yet reported. Our two males measure 22.4 and 22.1 mm SVL, respectively. They differ from both described species by more pronounced pedal webbing. One individual (ZMB 80870) exhibits very contrasting pattern on
flanks and back, not known to us to occur in *P. liberiensis* and not reported from *P. intermedius* either. The pair of white dorsal roundish dots occurs as well in *P. liberiensis*, however, there usually only in juveniles.

*Phrynobatrachus* sp. was associated with small creeks in swampy forest areas, dominated by *Raphia* palms. The males could be the unknown males of *P. intermedius*, although sex dependent differences in webbing would be unusual for the genus. In order to clarify the taxonomic situation molecular data are needed. Future work in south-western Ghana and south-eastern Ivory Coast should carefully examine *P. liberiensis* and *P. plicatus* to assess if *P. intermedius* (or other cryptic species) occur in the few remaining rainforests in these regions, and in particular to collect tissue samples and call recordings.

**Discussion**

During our survey we recorded 33 anuran species. The overall species richness of TESF was lower compared to species richness recorded in western Ivorian forest areas, for instance the Taï National Park (Ernst et al. 2006), Mont Sangbé National Park (Rödel 2003), or the Haute Dodo and Cavally Classified Forests (Rödel and Branch 2002), but similar to Mont Péko National Park (Rödel and Ernst 2003). Compared to these and other West African forest areas with known amphibian assemblages such as south-western Ghana (Rödel et al. 2005), south-eastern Guinea (Rödel et al. 2004) or north-western Liberia (Hillers and Rödel 2007), the TESF ranks among the areas of median to low amphibian species richness. Amphibian
surveys in two other eastern Ivorian forests, Yakassé-Mé village forest and Banco National Park, documented 24 and 28 species, respectively (Assemian et al. 2006, Kouamé et al. 2014b). It thus could be speculated that this region is comparatively species poor concerning amphibians. However, our estimations revealed that we probably do not yet know the entire amphibian fauna of the TESF. More intensive surveys, especially in areas and microhabitats not yet investigated, may result in an increasing number of species. Further species likely to be recorded in TESF are Acanthixalus sonjae, Hyperolius laurenti and H. viridigulosus. The occurrence of Cardioglossa occidentalis, Astylosternus laticepsalus, Leptopelis macrotis, Phryctimantis boulengeri, Ptychadena aequiplicata and P. supercilfer, seems possible because these species have been found in the Ankasa Conservation Area (Rödel et al. 2005), a rainforest in south-western Ghana and thus almost adjacent to TESF. Although we spent always the same number of hours per visit searching for frogs at the eight sites, the number of visits per site varied largely from 2–8 between sites (compare in Appendix 1). Therefore, the probability to record all species at a given site, and thus across different habitats, likely varied. Moreover, as the survey did not cover the entirety of the swamp forests, it is possible that further species occur in forest parts which were not studied. In contrast it is also possible that the documented forest alteration is responsible that some species, including the above mentioned ones, all being forest specialists, declined or even went extinct in our study site.

We recorded different subsets of the 33 anuran species in TESF. The highest species richness at site KA (21 spp.) was most probably due to the fact that this area was less altered and included more breeding sites, puddles, ponds, creeks, as well as thicker layer of leaf-litter than the remaining sites. More than half of the recorded species are restricted to West Africa, or to smaller parts of this region; the majority of these including all threatened species, are forest specialists. However, the records of many TESF species with wide distributional ranges, and broad habitat tolerance, clearly reflects altered forest conditions, due to deforestation and conversion of forests into palm plantations or rubber monocultures. The latter are steadily increasing in the eastern forest zone (Kouamé et al. 2014a). From western Ivorian forests it has been shown that logging has a serious effect on the composition of frog assemblages. Many forest specialists seem to be unable to prevail in degraded forests, most probably due to an altered microclimate with which they cannot cope (Ernst and Rödel 2005, Ernst and Rödel 2006, Hillers et al. 2008). Hence, exploring more of the potentially pristine areas in the southern parts of TESF is needed to get a complete impression concerning the presumed original anuran composition in TESF.

South-eastern Ivory Coast suffered from intensive deforestation and only a few forest remnants still prevail (Parren and de Graaf 1995, Bakarr et al. 2004, Zadou et al. 2011, Mayaux et al. 2013). This is worrying because the rainforests of this region and neighbouring western Ghana are supposed to have acted as a Pleistocene forest refugium (e.g. Parren and de Graaf 1995, Maley 1996), and thus may comprise a unique fauna and flora. This is especially true for amphibians (Penner et al. 2011), which however, are still far from being completely

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Figure 5. Dorsolateral and ventral view of a male (a, b; ZMB 80870) Phrynobatrachus sp. from the Tanoé-Ehy Swamp forests; compare text.
known. Therefore, further surveys are highly recom-
mended for all remaining forests, with a particular focus
on primary forest. Such intensified research would lead to
a better knowledge of the regional amphibian fauna,
population sizes and distribution pattern of particular
species. This would be especially important for species
with high conservation concern, such as *Phrynobatraca-
chus intermedius*, *Morrelia cyanophthalma* and other
rare or threatened endemics. Finally, further investiga-
tions could lead to a better understanding of the diver-
sity in VNRs which would help to provide further conser-
vation recommendations.

Acknowledgements

We are indebted to the “Centre Suisse de Recherche Sci-
entifique, Abidjan” in general, and the Research and Ac-
tion Program for the Conservation of Primates (RASAP-
CI) in particular for financial support and invitation to
participate with this amphibian survey. We are also
thankful to the Ivorian Ministry for Scientific Research
for permitting access to the Tanoé-Ehy Swamp Forests.
We are especially grateful for the collaboration from
chiefs and elders of the local communities surrounding
Tanoé-Ehy Swamp Forests. Local assistants and field
guides were of invaluable help during field work: their
hard work, dedication and their inspiring companionship
helped to make this expedition a success. We thank D.C.
Blackburn, J. Köhler and A. Channing for valuable com-
ments and corrections!

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Appendix 1

Geographic coordinates of the survey sites, search effort (measured in person-hours: p-h) and short habitat characterization.

| Sites | Latitude   | Longitude  | # of visits | p-h | Habitat description                                                                 |
|-------|------------|------------|-------------|-----|-------------------------------------------------------------------------------------|
| EB    | 05°20’742”N | 05°73’568”W | 2           | 28  | dense vegetation with large canopy gaps and thick shrubby undergrowth; river present |
| KA    | 05°11’177”N | 05°73’010”W | 5           | 70  | dense vegetation with large canopy gaps and thick shrubby undergrowth; river, creeks, numerous puddles and ponds present; thick leaf-litter layer |
| KW    | 05°18’292”N | 05°70’840”W | 3           | 42  | dense vegetation with large canopy gaps and thick shrubby undergrowth; swamp; flooded site |
| MK    | 05°18’482”N | 05°71’016”W | 3           | 42  | rice field; small scale subsistence farming; disturbed area; very large pond present |
| MN    | 05°16’755”N | 05°72’052”W | 8           | 112 | palm tree plantation; very large pond present |
| PN    | 05°16’938”N | 05°71’753”W | 6           | 84  | very uniform and relatively dry forest with dense canopy (no river, ponds or puddles present) |
| RE    | 05°11’181”N | 05°73’011”W | 5           | 70  | swamp forest dominated by Raphia palms, partly open, on the periphery of a village |
| YA    | 05°20’739”N | 05°73’565”W | 3           | 42  | grassland at the edge of the forest; creek present |

Appendix 2

List of amphibian voucher specimens from the Tanoé-Ehy Swamp Forests. Given are field (TFK) and collection (ZMB) numbers, collection site (compare Appendix 1) and collection date.

**Arthroleptidae:** *Arthroleptis* spp.: TFK001–TFK043 (RE, 25 June 2010); *Leptopelis* occidentalis: TFK044–TFK046 (KA, 2 July 2010); *L. spiritusnoctis*: TFK047–TFK053 (EB, 3 July 2010), TFK054–TFK061 (KA, 27 June 2010), TFK062–TFK068 (KW, 4 July 2010), TFK069–TFK072 (YA, 5 October 2010);

**Bufonidae:** *Amietophrynus* maculatus: TFK073 (KA, 17 June 2010), TFK074 (RE, 25 June 2010); *A. regularis*: TFK075 (EB, 26 June 2010), TFK076 (MN, 1 July 2010), TFK077 (RE, 4 July 2010);

**Dicroglossidae:** *Hoplobatrachus occipitalis*: TFK078–TFK084 (KA, 17 June 2010), TFK085–TFK089 (RE, 20 June 2010), TFK090 (YA, 23 September 2010);

**Hyperoliidae:** *Afrisaxicola dorsalis*: TFK091–TFK098 (EB, 26 June 2010), TFK099 (KA, 17 June 2010), TFK100 (MN, 23 June 2010), TFK101 (RE, 25 June 2010), TFK102–TFK105 (YA, 23 September 2010); *A. fulvovittatus fulvovittatus*: TFK106–TFK108 (RE, 3 October 2010); *A. nigeriensis*: TFK109–TFK112 (RE, 24 September 2010); *Hyperolius concolor concolor*: TFK113–TFK115 (EB, 03 July 2010), TFK116 (KA, 17 June 2010), TFK117 (KW, 22 June 2010), TFK118 (MK, 22 June 2010), TFK119 (MN, 25 June 2010), TFK120 (RE, 25 June 2010), TFK121 (YA, 3 July 2010); *H. fusciventris burtoni*: TFK122–TFK124 (EB, 26 June 2010), TFK125 (KA, 17 June 2010), TFK126 (KW, 22 June 2010), TFK127 (MK, 22 June 2010), TFK128 (MN, 23 June 2010), TFK129 (PN, 26 June 2010), TFK130 (RE, 25 June 2010), TFK131 (YA, 3 July 2010); *H. guttatus*: TFK132–TFK143 (KW, 22 June 2010), TFK144–TFK145 (MK, 26 June 2010), TFK146–TFK148 (MN, 28 June 2010), TFK149 (RE, 4 July 2010); *H. picturatus*: TFK150 (KW, 4 July 2010), TFK151–TFK154 (RE, 29 June 2010); *H. sylvaticus*: TFK155 (KA, 2 July 2010);

**Kassina arboricola:** TFK156–TFK159 (KA, 17 June 2010); *Morearella cyanothalamia*: TFK160 (MK, 22 June 2010), TFK161–TFK164 (RE, 4 July 2010), ZMB 80869 (RE, 17 June-29 July 2010);

**Phrynobatrachidae:** *Phrynobatrachus alleni*: TFK165–TFK171 (PN, 26–27 June 2010); *P. calcaratus*: TFK172–TFK175 (KA, 5 October 2010), TFK176–TFK178 (MK, 22 June 2010); *P. ghanensis*: TFK179–TFK191 (KA, 5 October 2010); *P. gutturosus*: TFK192–TFK197 (EB, 26 June 2010), TFK198 (KA, 5 October 2010), TFK199 (KW, 5 October 2010); *P. sp. (cf. intermedius)*: TFK200 (RE, 3 October 2010), ZMB 80870-80871 (RE, 17 June-29 July 2010); *P. latifrons*: TFK201–TFK207 (EB, 26 June 2010), TFK208–TFK212 (KA, 17 June 2010), TFK213 (KW, 4 June 2010), TFK214 (MK, 22 June 2010), TFK215–TFK219 (MN, 25–26 June 2010), TFK220 (RE, 25 June 2010), TFK221–TFK224 (YA, 5 October 2010); *P. liberiensis*: TFK226 (RE, 24 September 2010); *P. plicatus*: TFK227–TFK229 (KA, 17 June 2010);

**Pipidae:** *Silurana tropicalis*: TFK230 (RE, 3 October 2010); *Ptychadenidae:** *Ptychadena bibroni*: TFK231–TFK235 (EB, 26 June 2010), TFK236 (MN, 23 June 2010), TFK237 (YA, 3 July 2010); *P. longirostris*: TFK238 (EB, 26 June 2010), TFK239 (KA, 27 June 2010), TFK240 (RE, 4 July 2010); *P. mascareniensis*: TFK241–TFK249 (KA, 17 June 2010), TFK250 (MK, 19 June 2010), TFK251 (PN, 4 October 2010); *P. oxyrhynchus*: TFK252–TFK254 (KA, 17 June 2010); *P. pumilio*: TFK255 (KA, 17 June 2010), TFK256 (MK, 19 June 2010), TFK257 (MN, 21 June 2010), TFK258 (RE, 3 October 2010), TFK259 (YA, 5 October 2010);

**Pyxicephalidae:** *Aubria subsigillata*: TFK260–TFK261 (KA, 22 September 2010), TFK262 (RE, 24 September 2010), TFK263 (YA, 23 September 2010); *Ranidae:** *Hylarana albolabris*: TFK264–TFK265 (EB, 3 July 2010), TFK266 (KA, 2 July 2010), TFK267 (MN, 2 October 2010), TFK268 (PN, 4 October 2010), TFK269 (YA, 5 October 2010); *H. occidentalis*: TFK270–TFK271 (KA, 17 June 2010), TFK272 (MN, 18 June 2010), TFK273 (PN, 24 July 2010), TFK274 (RE, 3 October 2010).
The European medicinal leech *Hirudo medicinalis* L.: Morphology and occurrence of an endangered species

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Abstract

Although the European medicinal leech (*Hirudo medicinalis* L. 1758) is one of the best-known members of the Hirudinea due to its use in phlebotomy, this species has been confused with the Mediterranean taxon *H. verbana* Carena 1820. Here we describe the morphology of adult and juvenile *H. medicinalis* and document its genetic distance to *H. verbana*, using newly acquired mitochondrial DNA-sequence (cytochrome *c* oxidase subunit I, CO-I)-data from specimens collected in Germany. Our CO-I analysis shows that *H. medicinalis* and *H. verbana* differ by 9.4%. Hence, the original *Hirudo*-population diverged ca. 10 million years ago so that today two geographically separated biospecies exist that co-occur in only a few natural habitats. We analyzed the behaviour of adult *H. medicinalis*, but could not find differences with respect to its sister taxon *H. verbana*. Finally, we summarize the occurrence of *H. medicinalis* in Central Europe and conclude that this once widely distributed freshwater species largely disappeared in many countries. We suggest that the loss of natural freshwater ecosystems, with flat, warm banks, and amphibians (frogs, newts and toads) as preferred host organisms for the juveniles, are largely responsible for the decline of *H. medicinalis* in Northern Europe.

Key Words

Endangered species  
evolution  
*Hirudo medicinalis*  
medicinal leeches  
taxonomy

Introduction

Among the currently ca. 14 000 accepted species of Annelida (segmented worms) found worldwide in freshwater, marine and terrestrial ecosystems, Linnaeus (1758) first described the two most well-known forms: the earthworm (*Lumbricus terrestris*) and the medicinal leech (*Hirudo medicinalis*). However, this Linnaean system of the “classis Vermes”, with the three orders “Intestina, Mollusca and Testacea”, was soon replaced by Lamarck’s more detailed account of classification of the “lower animals” (Vinarski 2014). In Vol. 5 of a series of monographs on the systematics of invertebrates, Lamarck (1818) introduced the class Hirudina (leeches), and listed two European species, *Hirudo medicinalis*, and “*H. sanguisorba*”. While the first taxon, the type species of the class Hirudina Lamarck, 1818 (sanguis medicinale, i.e., the medicinal leech), is still accepted today as a valid species, Lamarck’s “*H. sanguisorba*” remains an enigma.

During subsequent decades, notably when the use of leeches in phlebotomy (bloodletting) became very popular throughout Europe (ca. 1850), numerous “varieties” of “*H. medicinalis*” were distinguished by naturalists as well as practitioners (Herter 1936, 1937).

In his classic monograph on leeches, Mann (1962) adopted this interpretation and wrote that *H. medicinalis* must be regarded as a highly variable species. Accordingly, Herter (1968) and Sawyer (1986), in their influential books, argued that all colour variants of European medicinal leeches should be assigned to the taxon *H. medicinalis* Linnaeus, 1758 (syn. *H. officinalis* Savigny, 1822; see also Hechtel and Sawyer 2002). However, based on detailed analyses of pigment patterns and DNA-sequences, it was documented that *H. officinalis* is not a “colour variant of Linnaeus’ type species”, but a separate taxon, the Mediterranean medicinal leech *H. verbana* Carena, 1820 (Nesemann and Neubert 1999; De Salle et al. 2005; Kutschera 2004, 2006, 2007, 2012a, 2012b; Trontelj et al. 2004; Siddall et al. 2007; Phillips and Siddall 2009; Elliott...
and Kutschera 2011; Elliott and Dobson 2014). Utevsky and Trontelj (2005) provided a key to all known European species in the genus *Hirudo*, and Kutschera (2012a, 2012b) summarized their geographical distribution.

In the present article, we describe the morphology of juvenile and adult *H. medicinalis*-individuals, add information on its evolutionary distance to its sister taxon *H. verbana*, and summarize observations on the behaviour, ecology and distribution of this endangered species.

**Materials and methods**

Adult and juvenile European medicinal leeches (*H. medicinalis*) (plus cocoons) were obtained from undisturbed habitats of eastern Germany (Elliott and Kutschera 2011), and specimens of Mediterranean medicinal leeches (*H. verbana*) were purchased from a commercial supplier (Sudak, Tr-59560 Murefte Tekirdag, Turkey) (Kutschera and Roth 2005). The leeches were kept in aqua-terraria (90 x 40 x 60 cm, depth of the pond water ca. 10 cm; temperature 22 to 26 °C), and observed/photographed alive. Specimens of *H. medicinalis* were killed by adding 80% ethanol to the water, so that the animals were preserved in their non-contracted, natural shape, and photographed. Extraction of DNA from part of the posterior sucker, sequencing of a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (CO-I), and phylogenetic analyses, based on newly acquired (and deposited) GenBank-data were performed as described (Kutschera et al. 2007, 2013; Wirchansky and Shain 2010).

**Results**

**Morphology of adult *H. medicinalis*-individuals**

Leeches are animals with an organization akin to that of earthworms, but having certain modifications associated with a predatory or parasitic mode of life. The limitation of the number of body segments facilitates a greater degree of agility than would be the case if the body was as long as that of most earthworms. The segments are each subdivided into a number of annuli, five in the Hirudinidae. There is some disagreement about the relationship between annulation and segmentation (Mann 1962). Externally, the annuli look much alike, and there is little indication of segmentation. Perhaps the best guide is the pattern of colouring, which often repeats itself once per segment. For example, a distinctive pattern separates *H. medicinalis* from its sister taxon *H. verbana* (Figs 1, 2). On the middle annulus of each segment are sensory papillae (Pap in Fig. 3A). These may be prominent, and are often marked by spots of light pigment. Papillae may also be present on other annuli of a segment. On the first few segments of the body, some of the sensory papillae are replaced by black-pigmented eye spots, five pairs of eyes arranged in a crescent in *Hirudo medicinalis* (four eyes are marked in Fig. 3B).

The size of the suckers relative to the body varies according to the mode of life of the leech species and, in *H. medicinalis*, the anterior sucker is quite small. The buccal cavity is lined by muscular ridges surmounted by cuticular teeth, and the mouth is a wide aperture occupying the whole of the anterior sucker (Fig. 3C). Following the pharynx is a region of the alimentary canal, the crop, which is dilated for the storage of food. In the sanguivorous *H. medicinalis*, it is drawn out into lateral arms referred to as diverticula.

The clitellum is situated towards the anterior of the body (Fig. 4A). The male reproductive aperture is median and unpaired. There are two internal ducts leading to it but these unite to form a single genital atrium with one external gonopore and a ‘tube-like’ male copulatory organ (Fig. 4B). The female pore is likewise median and unpaired, and is posterior to the male pore.
chiefly in July and August. Over one to 12 days, each mature leech will lay 1 to 8 cocoons with usually 12 to 16 eggs per cocoon; sometimes more, but with some infer-

Figure 2. Dorsal and ventral views of a representative, alcohol-preserved specimen of *H. medicinalis* collected in eastern Germany. The species-specific pigment patterns are visible.

Cocoons and juvenile *H. medicinalis*

Mature medicinal leeches leave the water to deposit their cocoons in a moist place just above the water line on the shore or bank. The spongy cocoons (Fig. 5A) are laid chiefly in July and August. Over one to 12 days, each mature leech will lay 1 to 8 cocoons with usually 12 to 16 eggs per cocoon; sometimes more, but with some infer-

Figure 3. Details of the midbody, in dorsal view (A), the head (B) and the anterior sucker (ventral view) (C) of an adult, alcohol-preserved *H. medicinalis*. As = anterior sucker.

Figure 4. Lateral view of an adult, alcohol-preserved *H. medicinalis* (A) and position of the male () and female () gonopores on the ventral side (B), with the tube-like male copulatory organ outside of the body.
tile eggs. In the laboratory, each adult laid 1 to 7 cocoons with 3 to 30 eggs per cocoon, and produced 2 broods per year under optimum conditions. Hatching time varied from 4 to 10 weeks, depending upon the temperature, and the live mass of each newly-hatched leeches (length: 8–12 mm) varied from 12 to 60 mg.

The markings of the juveniles are very similar to those of the adults except there is less pigment on the ventral surface (Figs 5, 6). Hatchlings can survive for up to 100 days without feeding, but fed leeches in the laboratory attained a live mass of 0.5 to 0.6 g at the end of their first year, about 1.4 g in their second, and about 2.4 g in their third year. Similar results were obtained for *H. verbana* (Kutschera and Roth 2006). Although there is a paucity of field information, it is generally agreed that *H. medicinalis* and *H. verbana* take at least two years to reach the breeding stage in the wild, and slow-growing leeches may not breed until they are three or four years old.

**Behaviour of *H. medicinalis* vs. *H. verbana* and hyper-parasitism**

Living, adult individuals of *H. medicinalis* and its sister species *H. verbana* were maintained in aqua-terraria. Despite the fact that the species were clearly distinguishable based on their pigment patterns on both the dorsal and ventral sides of their body (Fig. 1), qualitative observation of their behavioural patterns revealed no differences. For a large part of the year when water temperatures are low, medicinal leeches are quiescent and remain buried in the mud or under submerged objects at the edge of the pond. As water temperature increases, the leeches become very responsive to water disturbance caused by a potential host, and swim towards the source of blood. Laboratory experiments showed that 86 % and 95 % of unfed leeches responded to low-amplitude surface waves (about 1 mm high) by swimming, whilst only ca. 60 % of fed leeches displayed a reaction. The neurophysiology of this detection of water motion was described in detail by Friesen (1981).

Laboratory experiments have also shown that when a medicinal leech is near a mammalian host, such as the skin of a human, it uses heat detection, the optimum response occurring at 33 to 40 °C (Dickinson and Lent 1984), and also chemosensory stimuli (Elliott 1986), both receptors being located in the anterior end of the leech (Fig. 3B). The leech explores the outer cell layer of the host for a suitable feeding site, then pierces the skin with its three jaws armed with numerous sharp teeth, and finally sucks the blood of its host. We also observed that, in the wild and in the laboratory, *H. medicinalis* suck blood from amphibians, such as the edible frog (Fig. 7).

However, other leech species will sometimes feed on *H. medicinalis*. Young Glossiphonia complanata that were co-cultivated with medicinal leeches frequently obtained their first meal by feeding on the body of *H. medicinalis*. In a quantitative study in a tarn (= pond) in North-West England, *H. medicinalis* were found to be carrying all sizes of Helobdella stagnalis that were feeding on the host. The proboscis was inserted deep into the body wall of the host and the anterior portion of the body contracted regularly as fluid was extracted from the host, i.e., hyperparasitism was documented unequivocally. *H. stagnalis* did not kill its host or produce any obvious reactions. Similar observations were reported for *H. verbana* (Kutschera et al. 2010).
Phylogenetic analysis, divergence time and geographic distribution

In order to verify the taxonomic status of *H. medicinalis* from Germany (Figs 1 to 7), the DNA-barcoding approach was employed (De Salle et al. 2005). About half of the posterior sucker of ethanol-fixed individuals was excised and used for DNA-extractions (Fig. 6A, B). We obtained 625 base pair (bp)-sequences of the mitochondrial gene CO-I and compared our newly acquired data with those deposited in GenBank, a comprehensive database that contains publicly available nucleotide sequences (Benson et al. 2013). Our “German” sequences were found to be 100 % identical with two deposited CO-I-data for *H. medicinalis*, GenBank-Nos. AY786458 and HQ333519, obtained from individuals collected in France and Sweden, respectively (the latter corresponds to the Swedish Neotype of *H. medicinalis*, SMNH Type-8027; Kvist et al. 2010). Hence, populations of *H. medicinalis* in these three European countries display identical genetic signatures with respect to the CO-I-barcoding technique, and hence clearly represent one widely distributed biospecies.

Based on CO-I-sequences acquired in our laboratory for *H. verbana* and other leech species (Kutschera 2010, 2011; Kutschera et al. 2007, 2013), supplemented by additional GenBank CO-I-data, the results shown in Table 1 were obtained. The type species *H. medicinalis* and its sister taxon *H. verbana* differ by ca. 9.4 % with respect to CO-I-sequences. This distance indicates that *H. medicinalis* and *H. verbana* diverged ca. 10 million years ago (Wirchansky and Shain 2010), presumably via geographic isolation of one ancestral parent species, so that today two biospecies occur that occupy the Northern and Southern parts of Europe, respectively, with little overlap of the populations (Fig. 8A, B). The genetic distance of all the other leech species listed in Table 1 is ca. 17 to 26 %, documenting much larger divergence times.

Figure 7. Two adult, free-living *H. medicinalis* in the process of sucking blood from an edible frog (*Rana esculenta* L.). The amphibians usually survive these attacks (adapted from Manzke and Winkler 2012).
Discussion

The historical use, ecology, genetics and conservation of medicinal leeches was recently summarized (Elliott and Kutschera 2011; Elliott and Dobson 2014), and the present discussion is based in part on these extensive reviews. As noted in the Introduction, Hirudo medicinalis was once abundant in Northern Europe, from Ireland in the west to the Ural Mountains in the east, and from Southern Scandinavia to the countries bordering the Mediterranean (Elliott and Tullett 1984, 1986, 1992), where the Southern species H. verbana occurs (Fig. 8A, B). It is now rare throughout Western Europe, and endangered in many countries (Wells and Coombes 1987; Utevsky et al. 2008, 2010; Kovalenko and Utevsky 2012; Petrauskiené et al. 2011; Westendorf et al. 2008; Sawyer 2013a, 2013b; Trontelj and Utevsky 2005, 2012).

Large numbers of H. medicinalis were obtained from the wild in the 18th and early 19th centuries, and towards the end of this period, they were already scarce in many countries. This demand for medicinal leeches was not restricted to Europe. Hirudo medicinalis does not occur naturally in North America, and large numbers were imported from Europe into the United States in the 18th and 19th centuries. Several attempts were made to rear this species in the US, without positive results (Elliott and Kutschera 2011). As medicinal leeches became more difficult to find in the 19th century, the indigenous supply was supplemented by importations of other species of medicinal leeches from outside Western Europe. There was also the development of ‘leech farms’, especially in France and Germany. As late as 1890, a leech farm near Hildesheim in Germany was breeding between three and four million individuals per year (Herter 1968). Leech farms still exist today but, unfortunately, they often rely on imported leeches from Southeastern Europe and Turkey. These imports are often not H. medicinalis, but the closely-related

Table 1. Genetic distance between the type species of the Hirudinea, Hirudo medicinalis L. 1758, and other leeches, based on mitochondrial DNA-sequence data. The GenBank Accession Numbers for the mt-gene cytochrome c oxidase subunit I (CO-I) are added. AF = Africa, AS = Asia, EU = Europe, US = United States.

| Taxon                  | Locality   | GenBank Acc.-No. CO-I | Identity (%) |
|------------------------|------------|-----------------------|--------------|
| Hirudo medicinalis     | Sweden, EU | HQ333519              | 100          |
| Hirudo verbana         | Turkey, EU/AS | EF125043           | 90.6         |
| Haemopis sanguisuga    | Sweden, EU | AF462021              | 83.3         |
| Hirudinaria malinensis | Malaysia, AS | AY425449           | 82.4         |
| Erpobdella octoculata  | Germany, EU | AF003274             | 75.0         |
| Trocheta intermedia    | Germany, EU | DQ009669             | 74.1         |
| Glossiphonia complanata| Germany, EU | AF003277             | 77.7         |
| Helobdella californica | California/US | HQ686307            | 73.5         |
| Malagadella fallax     | Madagascar, AF | EF125044            | 79.5         |
| Xerobdella lecontei    | Austria, EU | EF125040             | 76.0         |

Figure 8. Geographical distribution of Hirudo medicinalis and H. verbana, based on data published in 2012 (A). In the species H. verbana, a western (w) and an eastern (e) phylogroup has been identified. Occurrence of medicinal leeches in the nest of aquatic birds (B). The photograph shows adult, living specimens of H. medicinalis (with cocoon, see Inset) collected from a nest of a water bird (western marsh harrier, Circus aeruginosus) in Poland (adapted from Kovalenko and Utevsky 2012 [A] and Buczyński et al. 2014 [B], respectively).
species, *H. verbana*, which has been confused with the ‘true’ medicinal leech (Michalsen and Roth 2006).

*H. verbana* was first described from Lago Maggiore in Northeast Italy (Latin: *Lacus Verbanus*) by Carena (1820) and later regarded as a sub-species of the European medicinal leech (*H. medicinalis ssp. officinalis*). Living, adult *H. medicinalis* and *H. verbana* (as well as the juveniles) have very distinct markings (Figs 1, 5). These two closely related leech species, which diverged ca. 10 million years ago from a common ancestor, were confused or both labelled as “*Hirudo medicinalis*”, until a close examination of their morphology, combined with breeding studies and DNA-sequencing experiments, yielded unequivocal proof that they are distinct, reproductively isolated taxa (Nesemann and Neubert 1999; Trontelj et al. 2004; De Salle et al. 2005; Kutschera 2004; 2006, 2007, 2012a, 2012b; Siddall et al. 2007; Phillips and Siddall 2009).

Earlier reviews of the literature on the ecology of *Hirudo medicinalis* showed that there was surprisingly little quantitative information on medicinal leeches in the wild and most of the numerical values were from laboratory studies (Mann 1962; Herter 1968; Elliott and Mann 1979; Sawyer 1986; Petrasukienė et al. 2011). Representative data, compiled from Davies and McLoughlin (1996), are summarized in Table 2. The typical natural habitat is a eutrophic pond with a muddy substratum, littoral vegetation, and a high summer temperature. It should also be a breeding site for amphibians (frogs, toads and newts). Although *H. medicinalis* and *H. verbana* are often reported as feeding almost exclusively on the blood of mammals (cattle, horses, deer, humans), they will also suck the blood of fish, water birds, and especially amphibians, both the adults and their larvae. Hoffman (1960) observed adult leeches feeding on toads (*Bufo bufo* L.) in April when the latter returned to ponds to breed. The leeches attacked both male and female toads, often when the male was grasping the female in the ‘nuptial embrace’. Large numbers of toads died from loss of blood and their corpses provided food for another leech species, *Haemopis sanguisuga*. Tadpoles as well as juvenile newts are especially important for young medicinal leeches that are unable to pierce mammalian skin for the first two feedings. We corroborated Hoffman’s (1960) findings that were extended by Benecke (2009), Manzke and Winkler (2012) and Winkler and Manzke (2014) (Fig. 7). These authors provided, together with Jueg (2009), evidence for the occurrence of as yet undiscovered relict-populations of *H. medicinalis* in Eastern Germany.

Six decades ago, laboratory studies showed that the preferred temperature of *H. medicinalis* in a gradient of 7 to 43 °C was 21 °C (Kaiser 1954), a value midway between the 50 % and 90 % active leeches, as documented in a more recent study (Elliott 2008). Optimum temperature ranges for growth (22 to 25 °C) and breeding (25.5 to 27.5 °C) in the laboratory were similar to predicted values for maximum activity in the field. At 39 to 43.5 °C, the upper lethal range is remarkably high for this species (Kaiser 1954). These high temperature requirements have important implications for the survival of *H. medicinalis* in the wild: the leeches were unable to reproduce and survive in many water bodies simply because of the low water temperatures (Elliott and Tullett 1986).

A number of explanations have been proposed for the loss of many populations of *H. medicinalis* in Northern Europe, and these should all be considered in combination. Extensive over-collecting for blood-letting in the nineteenth century is frequently blamed, but used leeches were regularly discarded into the nearest pond or stream and thus may have enabled the survival of this species in the countryside. Contemporary collecting for experimental biology, medical use and pharmaceutical needs is probably a serious threat because the leeches are destroyed, often in large numbers (Shain 2009). Although leech farms offer an obvious solution, this only works if the commercial suppliers actually rear leeches, rather than importing them and thereby reducing populations in the wild (Michalsen and Roth 2006).

A reduction in the availability of suitable vertebrate hosts is another possible reason for the decline in countries where troughs are now used instead of ponds for the watering of cattle and horses. Changes in land use not only caused the loss of ponds but also isolation of the remaining freshwater ecosystems, even to wild animals such as deer, and this may have contributed to a reduction in blood meals from this source. However, there are still many parts of Europe where wild animals such as deer are plentiful, and therefore the almost complete absence of *H. medicinalis* in these areas is not due to a lack of mammalian hosts.

Davies and McLoughlin (1996) proposed the plausible hypothesis that the declining abundance of field populations of the European medicinal leech could be the result of lower available energy for growth, reflecting leeches now feeding predominantly on amphibian blood of lower energetic value than mammalian blood. This conclusion was supported by slow-growing wild populations of leeches from Dungenes, UK. A serological test was positive for 128 blood meals and showed that most leeches were feeding on amphibian blood with smaller numbers feeding on fish and birds, and only one leech sucking mammalian blood (Wilkin and Scofield 1990, 1991a, 1991b). In a Lake District tarn, only the larger mature leeches (>3.5 g) had fed on mammalian blood, and the proportion of ma-

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**Table 2.** Life history variables and reproductive success (i.e., number of offspring per individual and life time) of *H. medicinalis*, cultivated under sub-optimal laboratory conditions (20 °C). The animals were subsisted on mammalian (bovine) blood (n = 30) (adapted from Davies and McLoughlin 1996).

| Parameter                              | Range  | Mean (± SE) |
|----------------------------------------|--------|-------------|
| Time (years) from hatching to death     | 1.3–2.3| 2±0.1       |
| Cocoons produced/individual            | 2–41   | 12±5        |
| Hatchlings/cocoon                      | 0–14   | 4±1         |
| Offspring produced/individual          | 13–97  | 45±13       |

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ture leeches feeding on mammals varied from 19 to 26 % among years (Elliott 2008). The most important sources of blood for all leeches in the tarn were probably amphibians, such as newts, frogs, toads and their tadpoles (Fig. 7). Therefore, the slow growth of the leeches could be partially caused by the scarcity of mammalian blood in their diet. Leeches were observed feeding on horses that had waded into the tarn. They never fed to satiation, as seen in the laboratory when offered bovine blood in a sausage skin. Soon after a horse left the water, the leech detached and rapidly crawled back into the water. In wild populations, satiated leeches were never found and it was concluded that the annelids were feeding a little and often, rather than to satiation. A similar conclusion was reached for the population at Dungeness (Wilkin and Scofield 1990, 1991a, 1991b).

Water temperature will also affect the growth of *H. medicinalis*. Fast-growing leeches that attained maturity after only 289 days were kept at a constant 20 °C (Davies and McLoughlin 1996) (Table 2). This is just above the threshold temperature of 19 °C for most leeches to be swimming and searching for a host in a Lake District tarn (Elliott and Tullett 1986). Water temperature in the tarn exceeded this value on only 100 to 120 days from April to September and was thus a limiting factor for feeding and growth. The high temperature requirements of medicinal leeches (*H. medicinalis* and *H. verbana*) impose limitations on their distribution and occurrence. Therefore, the absence of these species from many water bodies may be due partially to the relatively high temperatures required for swimming activity, feeding, growth and breeding, as well as the scarcity of mammalian hosts. It should be noted that there has been a loss of many small, shallow ponds throughout Western Europe, and these are often the ideal habitat for medicinal leeches, especially if they contain amphibian species and water birds. In South-eastern Poland, Buczyński et al. (2014) found adult, breeding *H. medicinalis*-individuals, inclusive of cocoons, in the nests of water birds (Fig. 8B). This finding is in accordance with earlier observations of Herter (1936), who suggested that medicinal leeches may feed on nestlings of aquatic birds, notably when they fall into the water and attract hungry leeches (Young et al. 1981). Buczyński et al. (2014) suggest that nests may be important secondary habitats for medicinal leeches, but more observations in aquatic ecosystems, where natural leech populations occur, are necessary to support this hypothesis. These observations indicate that man-made losses of habitat could be also responsible for the demise of some populations of *H. medicinalis* (Grosser 2004; Elliott and Kutschera 2011; Elliott and Dobson 2014; Utevsky et al. 2008, 2010; Kovalenko and Utevsky 2012).

Finally, we want to point out that, although the distinctive features between *H. medicinalis* and *H. verbana* are obvious (Fig. 1) and have been described repeatedly in the literature (Nesemann and Neubert 1999; Trontelj et al. 2004; De Salle et al 2005; Kutschera 2004, 2006, 2007, 2012a, 2012b; Siddall et al. 2007; Phillips and Siddall 2009; Elliott and Kutschera 2011), *Hirudo medicinalis* is still confused with *H. verbana* and other leech species. For instance, Reece et al. (2011) depicted an adult *H. verbana*, but labelled the individual as “*H. medicinalis*”. In March 2012, the company Leeches U.S.A. Ltd. (Westbury, NY) sold “*Hirudo medicinalis*” for a research project to be conducted in the Weisblat-lab at the University of California (Berkeley). However, an inspection of the first author of this article (U. K.) revealed that all the specimens used for behavioural studies were unequivocally *H. verbana* Carena 1820 (see De Salle et al. 2005 for a DNA barcoding-identification for these mis-labelled medicinal leeches). Starr et al. (2011), Sartor et al. (2013) and Russel et al. (2014) depicted leech species, and described the individual as “*H. medicinalis*”, but in reality the taxa were the distinctly related *Hirudinaria mallensis* and *Malagadella* sp., respectively (see Table 1).

Hence, despite the fact that the European medicinal leech is, in addition to the taxonomically diverse earthworm *Lumbricus terrestris* (James et al. 2010), one of the most popular known annelids, the unique phenotype of this beautiful “annelid with character” is still widely unknown, even among biologists.

**Acknowledgements**

We thank Mr. M. Aurich (Biebertaler Blutegelzucht, Germany) for the provision of living medicinal leeches and Mr. U. Manzke for the photograph depicted in Fig. 6. This project was supported by the Bundesministerium für Wirtschaft und Technologie (Förder-Kz.: 03 ESFH 021) and the Alexander von Humboldt-Stiftung (Bonn, Germany; AvH-Fellowship Stanford/2012 to U. K.).

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