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An integrative taxonomic approach to infer the systematic position of *Chalepotaxis* Ancey, 1887 (Gastropoda: Stylommatophora: Helicarionidae)

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**ABSTRACT**

In the absence of knowledge about its anatomy, the systematic position of the genus *Chalepotaxis*, which inhabits a large part of South East Asia, has been historically dubious. The genitalia, sole of the foot and the caudal region of the type species, *Chalepotaxis infantilis* (Gredler, 1881. Zur Conchylien-Fauna von China. III. Stück. Jahrbücher der Deutschen Malakozoologischen Gesellschaft 8, 110–128), are described, which place this genus into the Helicarionidae family. DNA markers (cytochrome oxidase subunit I [COI] and 28S rRNA genes) corroborate this assignment and unequivocally remove the species from the Camainae (=Bradybaenidae) and Ariophantidae, where it had previously been placed. Radular morphology of the type species is also re-described and illustrated.

**Introduction**

*Chalepotaxis* Ancey, 1887 is an eastern Asian land snail genus of controversial systematic position. Currently, two species are assigned to this genus, namely *Chalepotaxis infantilis* (Gredler, 1881), recorded from southern China, northern Vietnam and Laos, and *Chalepotaxis spadix* (Schmacker & Boettger, 1891) (including the subspecies, *Ch. s. cinctus* (Pilsbry, 1901), both known from Taiwan (Yen 1939; Hsieh et al. 2006; Schileyko 2011). The type species of the genus, *Ch. infantilis*, was originally described as a form of *Bradybaena similaris* (Rang, 1831), as its general shell characters, such as its small shell size (7–8 mm), thin shell wall, narrow umbilicus and a single brown spiral band, resemble *Bradybaena similaris*. (The name ‘infantilis’ refers to the sharp, not thickened or expanded, apertural margin.)

Shortly after the original description, Gredler (1884) published illustrations of the radular morphology, which showed no similarity with any other genera known at that time. Based on this character, Gredler (1884) excluded this species from *Helix* Linnaeus, 1758 (where *Bradybaena similaris* was assigned) and tentatively placed it in *Nanina* Gray, 1834. Habe (1943) described *Taiwanosiphona* (type species: *Lamprocystis spadix* Schmacker & Boettger, 1891) on the basis of the usual radula of the genus; according to him it is similar to the Indian *Sophina* Benson, 1859 (which he spelled as ‘Siphona’). Later, he synonymised *Taiwanosiphona* with *Chalepotaxis* (Habe 1945). Based on the radula of *Sophina*, Blanford and Godwin-Austen (1908) erected the monotypic subfamily Sophininae. This taxon was most recently used at the tribe level (Sophinini, family Ariophantidae) by Schileyko (2003), who included several additional genera in Sophini without regard to the radular morphology.

In the absence of anatomical information, the systematic position of *Chalepotaxis* remained unresolved. Based only on conchological characters, some authors have placed it in the Bradybaenidae (Yen 1939; Schileyko 2004, 2011), whereas others (e.g., Hsieh et al. 2006) assigned the genus to the Helicarionidae. Based on the radula, a relationship with *Sophina* (Ariophantidae) might also be possible.

Here we present the genital anatomy, re-describe the radular morphology of the type species of this genus (*Ch. infantilis*) and discuss its systematic position. Based on DNA markers we sought to test its phylogenetic affinities with the Bradybaenidae, Helicarionidae and/or Ariophantidae and, as far as it is possible on the basis of available sequence data, to infer its position within the Stylommatophora.

**Materials and methods**

Radulae were directly observed without coating under a low-vacuum scanning electron microscope (Miniscope TM-1000, Hitachi High-Technologies, Tokyo). Individual buccal masses were removed and soaked in 2 M KOH solution for approximately 5 hours before extracting the radula, which was preserved in 70% ethanol.

**DNA extraction and sequencing**

In DNA marker selection the prime criterion was that the three potential families, Helicarionidae,
Ariophantidae and Bradybaenidae, should be well represented among publicly available sequences. As well as the standard barcoding marker, the mitochondrial cytochrome oxidase subunit I (COI) (Hebert et al. 2003), we decided to use the large subunit of the nuclear rRNA gene (28S). Evolving more slowly than COI, it reveals deeper phylogenetic relationships. Its relevance in stylommatophoran phylogeny was demonstrated by Wade et al. (2001, 2006).

DNA was extracted from the foot tissue using a First-DNA all tissue kit (Gen-ial, Troisdorf, Germany) following the manufacturer’s protocol. For the COI we used the LCO1490 (Folmer et al. 1994) and H2198-Alb (Gittenberger et al. 2004) primers and the same polymerase chain reaction protocol as Harl et al. (2014). For the 28S the LSU-2/LSU-5 primer pair (Wade et al. 2006) was used according to the protocol in Kruckenhauser et al. (2014). Successfully amplified products were sequenced at LGC Genomics (Berlin, Germany). The resulting COI and 28S fragments are 655 base pairs and 534 base pairs long, respectively (GenBank accession numbers are: KX027275 and KX027276).

### Phylogenetic analysis

A BLASTn (megablast) search was performed against the GenBank nucleotide database (http://blast.ncbi.nlm.nih.gov/) to identify the most similar entries, which were downloaded and used for further analyses. Alignment of COI sequences was unambiguous. For 28S we used the online version of MAFFT (Katoh and Standley 2013, http://mafft.cbrc.jp/alignment/software/) with the following settings: G-INS-i iterative refinement algorithm, gap opening penalty = 1.53, offset value = 0.123. The length of the resulting 28S alignment was 567 base pairs.

We used jModelTest2 (Darriba et al. 2012) to select the best-fit models of molecular evolution. The GTR + I + G model was selected for both data sets, which were then analysed separately because of the poor overlap of their taxa. Maximum likelihood (ML) analysis was performed by the GARLI web service hosted at http://molecularevolution.org (Bazinet et al. 2014), which used GARLI 2.1 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). After 40 independent runs from random starting positions we selected the trees of the best ML scores, and nodal supports were assessed by 100 bootstrap replications.

### Abbreviations

NHMW—Naturhistorisches Museum Wien (Vienna, Austria)

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**Results**

**Taxonomic account**

**Family Helicarionidae** Bourguignat, 1883

Chalepotaxis Ancey, 1887

Chalepotaxis Ancey, 1887: 22.

Taiwanosiphona Habe, 1943: 93.

Type species: Helix similaris var. infantilis Gredler, 1881, by original designation.

**Diagnosis**

Shell small (height: 4–5 mm, diameter: 6–7 mm), depressed conical; of about six whorls, fragile, glossy; sculpture weak, consists of low growth lines; aperture lunate with sharp peristome; umbilicus narrow. Foot long, slender, with caudal horn. Atrium very long, penis very long, partly covered by a sheath; epiphallus shorter than penis, with caecum in middle; retractor muscle inserts on caecum; bursa copulatrix with thickened base, short stalk and elongated bursa. Radular teeth with characteristically rounded, fan-shaped lower part and smaller triangular upper part.

**Chalepotaxis infantilis** (Gredler, 1881)

Helix similaris var. infantilis Gredler, 1881: 111.

**Material examined**

China, Hunan (湖南), Zhangjiajie Shi (张家界市), Wulingyuanqu (武陵源区), Guanyindong (观音洞), 29°22.90′N, 110°36.80′E (locality code: 20100508A), leg. Ohara, K., Okubo, K., Otani, J.U., 08.05.2010., NHMW 111548 (ex coll. J.U. Otani) (Figures 1–4).

**Anatomy** (Figures 2–3)

Two specimens were anatomically examined. Sole of foot slender, elongate, holopod (Figure 2A); lateral zones dark grey in colour, central zone light and sharply delimited (Figure 2A–B); caudal horn well developed, but blunt (Figure 2B–E).

Right ommatophoral retractor crossing penis and vagina. Atrium very long, thick; penis very long, roughly the same diameter throughout, except for distal portion, being partly covered by penial sheath;
inner wall of distal portion of penis lacking notable sculpture, that of proximal penial part with fine granules. Epiphallus much shorter than penis (approximately one-third length), with caecum at mid-point; slender, moderately long retractor muscle attached to epiphallus caecum and inserts in diaphragm. Proximal part of epiphallus internally with thickened, main fold, and several (at least 12) parallel, slender folds. Vas deferens long, relatively thick; a part of vas deferens connected to penial sheath by weak fibres. Bursa copulatrix with thickened base, relatively short stalk and elongated bursa; vagina relatively long, but shorter than penis; vaginal wall with granular surface in one specimen, smooth in the other. Spermoviduct swollen with uterus portion jelly-like. Proximal section of genitalia, as well as pallial region, of examined specimens missing.

Radula (Figure 4)
Jaw not found. Radula elongate, teeth arranged in V-shaped rows with apex pointing posteriorly; central tooth present, other teeth, 24 in number, not differentiated as laterals and marginals, although most marginally situated teeth smaller and narrower; each tooth with elongated plate consisting of larger, rounded, fan-shaped lower part and smaller, triangular, rather pointed upper part in lateral teeth, bicuspid in central teeth.

Molecular phylogeny
BLAST search did not find identical or highly similar COI entries. The most similar COI sequence (Stanisicarion freycineti (Férussac, 1821), Helicarionidae, EF015415) showed only 89% identity with Ch. infantilis under 98% query cover, whereas the first two sequences under 100% query cover were Bekkochlamys sp., EF015436 and Ovachlamys fulgens (Gude, 1900), EF015437 (87% and 86% identity, respectively). The first hundred most similar sequences (85%–87%) included sequences of various stylommatophoran taxa from the Helicarionidae, Gastrodontidae, Boettgerillidae, Vertiginidae, Bulimulidae, Milacidae, Agriolimacidae, Rhytididae, Pyramidulidae, Pristilomatidae, Limacidae, Streptaxidae and Arionidae.

The ML analysis of this data set resulted in a tree where Ch. infantilis shows affinity to some Australian, Japanese and Mascarene helicarionid species (namely Nitor circumcincta (Cox, 1868) EF015417, Mysticarion porrectus (Iredale, 1941) EF015413, Stanisicarion freycineti EF015415, Echonitor cyrtochila (Gude, 1905) EF015419 and Westracyjtis lissus (Smith, 1894) EF015420 from Australia, Caldwellia angularis (Férussac, 1821) EF015421 from Mauritius and Ovachlamys fulgens EF015437 from Japan, see Hyman et al. 2007). Nevertheless the bootstrap support of this clade was low.

In the 28S sequence, the BLAST search located five highly similar (> 95%) entries. Three of these (Plegma caelatura (Férussac, 1821) AY014103, Harmogenanina
argentea (Reeve, 1852) AY014101 and Fastosarion brazieri (Cox, 1873) AY014099) belong to Helicarionidae and two (Ratnadvipia sp. AY841312 and Euplecta gardeneri (Pfeiffer, 1846) AY841311) belong to Ariophantidae. In the ML tree, Ch. infantilis and the three above-mentioned helicarionid taxa form a monophyletic clade, though with only a modest bootstrap support (0.78) (Figure 5).

It is important to note that no bradybaenids or other members of the superfamily Helicoidea were among the first hundred most similar COI and 28S sequences.

Discussion

The presence of the caudal horn and the epiphallic caecum places Chalepotaxis in the limacoid clade (Hausdorf 1998; Wade et al. 2001). The genital anatomy of Chalepotaxis shows the greatest similarity with species of other South East Asian ‘helicarionoid’ genera, such as Petalochlamys Godwin-Austen, 1907 (type species: Macrochlamys formosana var. hypograpta Pilsbry & Hirase, 1908), Bekkochlamys Habe, 1957 (type species: Macrochlamys perfragilis Pilsbry, 1901) and Ovachlamys Habe, 1947 (type species: Macrochlamys fulgens Gude, 1900). These genera are traditionally classified in the Helicarionidae (Azuma 1982; Hsieh et al. 2006). On the other hand, Schileyko (2002) classifies Bekkochlamys and Ovachlamys in the Euconulidae.

Figure 3. Genital anatomy of Chalepotaxis infantilis (two different specimens). Note that the bursa copulatrix of the specimen B was lost during dissection. Abbreviations: a—atrium; bc—bursa copulatrix; de—distal part of epiphallus; e—epiphallus; ec—epiphallic caecum; p—penis; pe—proximal part of epiphallus; ps—penial sheath; rm—retractor muscle; so—spermoviduct; v—vagina, vd—vas deferens. Scale bar = 1 mm.

Figure 4. Radula of Chalepotaxis infantilis. A, Whole radula. B, Central and first lateral teeth. C, Marginal teeth. The enumeration of lateral teeth indicates their position in a row. C refers to the central tooth.
Petalochlamys in the Heli-}

carionidae. Resolving the conflict of the systematics
of the above-mentioned genera is beyond the scope
of this paper. Here we classify these genera as
helicarionids.

The species that is most similar to
Chalepotaxis in terms of genital anatomy is
Petalochlamys formosana (Schmacker & Boettger, 1891) (see Schileyko 2002),
which inhabits Taiwan. This species differs from
Ch. infantilis by the presence of a vermiform flagellum
and a penial sheath that covers a larger portion of
the penis. Otherwise, it is very similar in terms of the retractor
corer inserting on the epiphallar caecum and the
thickened proximal part of the epiphallus with a prominent
inner axial pilaster (Schileyko 2002). The type
species of Bekkochlamys (Bekkochlamys perfragilis
(Pilsbry, 1901) agrees with Chalepotaxis in genitalia
(Habe 1957; Azuma 1982), with the exception of the presence of a flagellum. Most other Bekkochlamys
species, namely Bekkochlamys dulcis (Pilsbry, 1902),
Bekkochlamys koshikijimana (Pilsbry & Hirase, 1904),
Bekkochlamys masakii (Kuroda, 1960), Bekkochlamys ter-
amachii Kuroda & Minato, 1976 and Bekkochlamys
septentrionalis (Jacobi, 1898) possess a flagellum,
which is therefore the usual condition for the genus.

On the other hand, Bekkochlamys micrograpta
(Pilsbry, 1900) and Bekkochlamys kurodai (Habe, 1957)
lack the flagellum, which is characteristic for Ch. infanti-
ilis (Habe 1957; Azuma 1982). Except for the lack of an
epiphalliac caecum, which is prominent in Chalepotaxis,
the anatomy of Ovachlamys kandai (Habe, 1957; Azuma 1982).
Other possibly related genera, such as Nipponochlamys
Habe, 1945, Japanochlamys Habe, 1946, Yamatochla-
mys Habe, 1945 and Takemasaia Azuma & Minato,
1976, differ from Chalepotaxis by possessing a penial
caeicum. On the other hand, both the flagellum and
the epiphalliac caecum have been lost secondarily in
Helicarioidea (Hausdorf 1998), therefore these char-
acter states are rather less important taxonomically.

The radula of Chalepotaxis is very characteristic and
differs from all other probable relatives, which usually
possess tricuspid central teeth and bi- or unicuspid
marginals, all with pointed cusps (Habe 1945, 1946).
In terms of the morphology of the teeth, the similarity of Chalepotaxis with the Indian Sophina (Ariophantidae)
can best be explained by parallel evolution, and the two genera are probably not closely related. The systematic position of *Sophina* in the Ariophantidae seems to be clearly established due to the prominent saccorbellum (Stoliczka 1871). It should be noted that the subfamilies Garnierinae and Neninae (Clausiliidae) show radular tooth morphology that is strikingly similar to that of *Chalepotaxis* (Loosjes and Loosjes-van Bemmelen 1973; Szekeres 1998). We suggest that future research should focus on the adaptive and systematic value of this trait in multiple land snail groups.

The molecular markers concordantly refute the possibility of a relationship of *Ch. infantilis* to the Bradybaeniidae. With the lack of highly similar sequence entries in public databases, the standard barcoding marker (COI) cannot provide unequivocal evidence for the relationships with Helicarioidae, but the result of 28S analysis is more indicative. This newly reconstructed 28S tree cannot be compared directly to that of Wade et al. (2006), because we included different taxa (the most similar sequences of the BLAST search) and we used only the 28S part of the ribosomal complex. Nevertheless, this tree topology is similar to that of Wade et al. (2006) in the sense that ‘limacoids’ form a monophyletic clade and also that taxa have a similar topology within this group. Placing *Chalepotaxis* into this framework demonstrates its obvious affinity to ‘limacoids’ (in the understanding of Wade et al. 2006) and shows that the closest relationship is with helicarioid genera.

In summary, both anatomical and molecular results place *Chalepotaxis* in a group that is generally called Helicarioidae. The closest relatives of *Chalepotaxis* are probably *Petalochlamsys*, *Bekkochlamys* and *Ovolachlamys*, which are distributed in the same geographical region. The relationship of *Chalepotaxis* with the Bradybaeniidae (=Camaenidae, see Wade et al. 2007) is not supported by our results.

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Disclosure statement

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