

Original Article

Phylogenetic insights into the terrestrial snails Helicoidei (Gastropoda: Stylommatophora) with special emphasis on the Camaenidae

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ABSTRACT

The superfamily Helicoidei are a widely distributed and diverse infraorder of land snails and slugs. Here, we constructed a phylogenetic tree based on sequences of the large subunit ribosomal RNA gene to investigate the relationships within the Helicoidei. Based on our phylogenetic tree, the family Camaenidae, as currently recognized, is not monophyletic, with the Polygyridae falling within the group. Likewise, the family Hygromiidae as currently defined is not monophyletic and incorporates the Geomitridae. The family Xanthonychidae form a monophyletic group that is sister to the Pleurodontidae. Likewise, the family Helicidae form a monophyletic group; it is sister to the branch incorporating Hygromiidae, Geomitridae, Pleurodontidae, Helicodontidae, and Xanthonychidae. The Solaropsidae are the sister group to a clade comprising other Sagdoidea. Our findings suggest that the Hygromiidae *s.l.* families should be restored to their previous name, Hygromiidae. A new genus of Bradybaeninae, *Jialiaous* Zhang gen. nov., is described here, with a simple genital system, single dart sac with multi-branched mucous glands, and a penis with a penial sheath.

Keywords: Helicoidei; Helicoidea; Camaenidae; China; molecular phylogeny; cladistics

INTRODUCTION

The Helicoidei Rafinesque, 1815 are a widely distributed and diverse infraorder of land snails and slugs distributed in Asia, Australia, North and South America, Europe, and Africa. This infraorder includes two superfamilies, Helicoidea Rafinesque, 1815 and Sagdoidea Pilsbry, 1895 (Bouchet *et al.* 2017).

Early studies on the taxonomy of the infraorder Helicoidei (known as the superfamily Helicoidea in classifications before Bouchet *et al.* 2017) were based on morphology. Thiele (1931) divided the Helicoidea into three families, the Bradybaenidae, Camaenidae, and Helicidae. Zilch (1960) followed Thiele's classification, and Taylor and Sohl (1962) introduced two new families to the Helicoidea, the Oreohelicidae (now Punctoidea) and Helminthoglyptidae. Boss (1982) removed the Oreohelicidae but added Ammonitellidae (now Rhytidoidea) to the Helicoidea. Tillier (1989) used quantitative methods to construct a parsimony tree of the Helicoidea based on morphological characters. He added six new families to the Helicoidea, the Helminthoglyptidae, Vitrinidae (now Limacoidea), Helicarionidae

(now Limacoidea), Polygyridae, Sagdidae, and Haplotrematidae (now Haplotrematoidea), and suggested that the Camaenidae might be a polyphyletic group. However, his work was challenged by Emberton and Tillier (1995), who disagreed with his definition of some morphological characters. Schileyko (1991) based his taxonomic system on genital characters. His Helicoidea *s.l.* included the superfamilies Helicoidea (which included the families Helicidae, Humboldtianidae, and Elonidae) and Xanthonychoidea [which included the families Helicostylidae, Bradybaenidae, Xanthonychoidea, Epiphragmophoridae, Helminthoglyptidae, Ciliellidae, Helicarionidae (now Helicarionoidea), and Hygromiidae]. In 1998, Schileyko (1998) raised the family Sagdidae to the superfamily Sagdoidea, comprising a single family, Sagdidae.

Molecular data have challenged the traditional morphology-based taxonomy of the Helicoidei (formerly Helicoidea in classifications before Bouchet *et al.* 2017). The first molecular study on the Helicoidea was conducted by Wade *et al.* (2001), who showed that the superfamily Helicoidea were a

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monophyletic group. Their phylogeny included seven families: the Helminthoglyptidae, Helicidae, Polygyridae, Camaenidae, Bradybaenidae, Hygromiidae, and Helicellidae. However, the family Camaenidae as traditionally defined (Pilsbry 1985) were not monophyletic (Wade *et al.* 2001). Following the results of Wade *et al.* (2001), Bouchet and Rocroi (2005) divided the Helicoidea into 19 families. They still considered the Camaenidae as a family but placed the American camaenids in the Pleurodontidae, following the opinion of Cuzzo (2003). Wade *et al.* (2006) and (2007) conducted further molecular analyses on the Helicoidea, which confirmed the non-monophyly of the Camaenidae. They also showed that the Camaenidae from America were not a single group but rather split into three distinct lineages: one clustering with the Hygromiidae and the others clustering with the Sagdidae. The Camaenidae from Asia/Australia in the study by Wade *et al.* (2007) clustered with the Bradybaenidae. Thompson and Naranjo-García (2012) introduced a new family of Mexican slugs, the Echinichidae, which were previously included in Xanthonychidae *s.l.* (= Xanthynchoidea), to the Helicoidea. Sei *et al.* (2014) found that the Trichodiscinidae clustered with Pleurodontidae rather than Xanthonychidae, and Trichodiscinidae were subsequently separated from the Xanthonychidae by Bouchet *et al.* (2017). Razkin *et al.* (2015) then reorganized the classification of the Hygromiidae, splitting it into three monophyletic families: Canariellidae, Geomitridae, and Hygromiidae. They also placed the Cochlicellidae as a tribe within the Geomitridae. Sei *et al.* (2017) proposed a major revision of the Helicoidea based on a comprehensive molecular phylogeny. They described a new family, the Labyrinthidae, belonging to the Helicoidea and expanded the Sagdoidea to include the families Sagdidae, Solaropsidae, and Zachrysiidae. Calcutt *et al.* (2020) also supported the grouping of these three families in the Sagdoidea, but the internal topology differed from that of Sei *et al.* (2017).

The taxonomy of the Helicoidei has been revised several times in recent years, with the most up-to-date taxonomy and nomenclature provided by Bouchet *et al.* (2017). One of their most significant changes was the merger of the Bradybaenidae and Camaenidae into a single family, named the Camaenidae, based on the phylogenetic studies by Scott (1996), Wade *et al.* (2006), and Wade *et al.* (2007), which showed that the Camaenidae were not a natural group, with the American Camaenidae being more closely related to Sagdidae or Hygromiidae than to other Camaenidae and with the Asian and Australian Camaenidae being more closely related to the Bradybaenidae. Gittenberger *et al.* (2012) and Bouchet *et al.* (2017) agreed that the Bradybaenidae should be considered a synonym of the Camaenidae. Another change was to move the Echinichidae, a group of slugs from Mexico, from the superfamily Xanthynchoidea to the Helicoidea (Bouchet *et al.* 2017). Bouchet *et al.* (2017) also merged five families that formed a clade [Epiphragmophoridae, Helminthoglyptidae, Humboldtianidae, Monadeniidae, and Xanthonychidae (based on Kohler's unpublished phylogenomic tree)], treating them as one family, the Xanthonychidae. Bouchet *et al.* (2017) also placed the Halolimnohelicidae under the Hygromiidae and accepted two new families, Canariellidae and Geomitridae, following Razkin *et al.* (2015). Finally, Bouchet *et al.* (2017) created an infraorder, the Helicoidei, which contained two superfamilies, Helicoidea and Sagdoidea. The Sagdoidea

included the American camaenid snails that clustered with Sagdidae in the phylogenetic tree of Wade *et al.* (2007) (Sei *et al.* 2017). The other American camaenid group, including *Theliodomus* and *Pleurodonte* and sister to the Hygromiidae in the tree of Wade *et al.* (2007), are now treated as Pleurodontidae (Bouchet *et al.* 2017).

Following dependence on shell characters and before the application of molecular methods, the taxonomy of the Camaenidae was based on the presence or absence of darts and associated morphological features in the genital system. The Camaenidae were based on the lack of darts and associated organs, whereas the Bradybaenidae were recognized by possession of dart sac(s) and related organs, such as mucous glands. However, these criteria do not reflect the relationships of these snails, based on molecular phylogenetic studies. Wade *et al.* (2007) found that the family Bradybaenidae was not a monophyletic group, and consequently, Gittenberger *et al.* (2012) treated Bradybaenidae as a synonym of Camaenidae. The current Camaenidae (following revisions by Bouchet *et al.* in 2017) form a diverse family of snails that live in various habitats in Asia and Australasia (Scott 1996, Bouchet *et al.* 2017) that consists of four subfamilies, the Camaeninae, Bradybaeninae, Hadrinae, and Helicostylineae. The Camaeninae were the first subfamily to be defined by Pilsbry (1895) as helices without a dart apparatus, with *Camaena* Pilsbry, 1895 as the type genus. The Camaeninae are distributed in East Asia and Southeast Asia. The subfamily Bradybaeninae were later established for many Chinese bradybaenid species by Pilsbry (1934) as helices that possessed dart structures, with *Bradybaena* Pilsbry, 1934 as the type genus. The Helicostylineae were introduced by Ihering (1909) for snails that possess a dart sac and are restricted to the Solomon Islands, the Philippines, and New Guinea, with type genus *Helicostyla* Férussac, 1821. The Hadrinae were proposed by Iredale (1937) for Australian helicid species without dart sacs and were based on the type genus *Hadra* E. von Martens, 1860. Bouchet *et al.* (2017) considered both the Rhagadinae and Sinumeloninae, which were previously recognized as subfamilies of Camaenidae (Bouchet and Rocroi 2005), as synonyms of the Hadrinae.

Morphological characters, including genital characters, are considered unstable characters, with several studies revealing incongruences between morphological and molecular approaches (e.g. Hirano *et al.* 2014). Moreover, some species that were classified based on shell characters have been found to have different genital features than were predicted from their shell characters. For instance, *Trichelix diminuta* [= *Moellendorffia diminuta*], which was formerly assigned to the Bradybaenidae, lacks a dart sac (Sutcharit *et al.* 2020), whereas *Traumatophora*, which was previously placed in Camaeninae, possesses a dart sac (Wu 2019). These are two examples of revised generic assignments based on genital characters that are consistent with molecular evidence but not with attributions based solely on shell characters.

To date, work on Chinese Camaenidae has been limited. Wu and Guo (2003) and Wu (2004) undertook morphological studies of the Chinese Camaenidae, and Wu (2019) introduced several useful genital characteristics. However, there is a lack of consistency in the use of terminology. For example, the sac opposite the vagina in the study by Wu (2004) is named the proximal accessory sac by Wu (2019) and Zhang *et al.* (2021a).

Table 1. Helicoidei specimens used in this study.

Vaught family/ subfamily	MolluscaBase family/ subfamily	Species	Collection/location	Collector	GenBank accession
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Acusta despecta chinensis</i> (Sowerby, 1839) ^a	Japan	S. Chiba	AY841337, PP069321
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Ainohelix editha</i> (A. Adams, 1868) ^a	Shimamaki, Hokkaido, Japan	S. Chiba	AY841338, PP069323
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Bradybaena similis</i> (Férussac, 1821) ^a	Sri Lanka	P. Karunaratne	MN022676, PP069329
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Cathaica pyrrhizona</i> (Phil- ippi, 1845) ^b	Dezhou City, China	Z. Zhang	PP069372
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Chosenelix impotens</i> (Pilsbry & Y. Hirase, 1908) comb. nov. ^b	Tianmenshan, China	G. Zhang	PP069378
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Euhadra amaliae</i> (Kobelt, 1875) ^a	Osaka City, Japan	P. Callomon	AY014140, PP069345
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Ezohelix gainesi</i> (Pilsbry, 1900) ^a	Sapporro, Hokkaido, Japan	S. Chiba	AY841339, PP069347
Not Applicable	Camaenidae: Bradybaeninae	<i>Jialiaous tchefouensis</i> (Crosse & Debeaux, 1863) ^b	Kunyushan, China	G. Zhang	PP069379
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Landouria radleyi</i> (Jousseau, 1894) ^b	Horton plain, Sri Lanka	F. Naggs	PP069359
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Manchurohelix twenhuaensis</i> (Ping & Yen, 1933) ^b	Jinlin, Jilin Province, China	G. Zhang	PP069337
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Mandarina ponderosa</i> (Pilsbry, 1901) ^a	Hahajima, Bonin Islands, Japan	S. Chiba & A. Davison	AY841320, PP069361
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Metodontia yantaiensis</i> (Crosse & Debeaux, 1863) ^b	Xuecheng, Zaozhaung, Shandong Province, China	G. Zhang	PP069389
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Nesiohelix bipyramidalis</i> Kuroda & Emura, 1943 ^a	Ryukyu, Japan	S. Chiba	AY841341, PP069369
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Nipponochloritis bracteatus</i> (Pilsbry, 1902) ^a	Sendai, Japan	S. Chiba	AY841319, PP069370
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Paraegista takahidei</i> Kuroda & Azuma, 1951 ^a	Hokkaido, Japan	S. Chiba	AY841340, PP069371
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Plectotropis vulgivaga</i> (Schumacher & Boettger, 1890) [= <i>Aegista vulgivaga</i>] ^a	Osaka City, Japan	P. Callomon	AY014139, PP069322
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Pliocathaica buwigneri</i> (Deshayes, 1873) ^b	Huanxian, Qingyang, Anhui Province, China	Unknown	PP069363 ^c
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Pseudiberus zenoi</i> (Gredler, 1882) ^b	Jinan, China	G. Zhang	PP069326
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Pseudobuliminus dongyiicus</i> ^b	Lushan, China	G. Zhang	PP069358
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Trishoplita hachijoensis</i> (Pilsbry, 1902) ^a	Nijijima Island, Izu Is- lands, Japan	S. Chiba	AY841345, PP069387
Bradybaenidae: Bradybaeninae	Camaenidae: Bradybaeninae	<i>Virginhelix virgo</i> (Pilsbry, 1927) ^b	Guichuan Road, Beijing City, China	G. Zhang	PP069360
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Coniglobus mercatorius</i> (Pfeiffer, 1854) ^a	Kikai Island, Ryukyu, Japan	S. Chiba	AY841324, PP069340
Camaenidae: Camaeninae	Camaenidae: Bradybaeninae	<i>Satsuma japonica</i> (Pfeiffer, 1847) ^a	Osaka City, Japan	P. Callomon	MN022674, PP069381
Camaenidae: Camaeninae	Camaenidae: Camaeninae	<i>Amphidromus</i> sp. ^a	Unknown	D. Reid	AY841317 & AY841318, PP069332 ^c
Camaenidae: Camaeninae	Camaenidae: Camaeninae	<i>Beddomea albizonata</i> (Reeve, 1849) ^b	Homadola, Sri Lanka	D. Raheem	PP069328

Table 1. Continued

Vaught family/ subfamily	MolluscaBase family/ subfamily	Species	Collection/location	Collector	GenBank accession
Camaenidae: Camaeninae	Camaenidae: Camaeninae	<i>Trichelix diminuta</i> (Pilsbry & Y. Hirase, 1905) [= <i>Moellendorfia diminuta</i>] ^a	Ryukyu, Japan	S. Chiba	AY841329 & AY841330, PP069367 ^c
Camaenidae: Camaeninae	Camaenidae: Hadrinae	<i>Sphaerospira fraseri</i> (Gray in Griffith & Pigeon, 1833) ^a	Brisbane, Queensland, Australia	J. Stanisic	AY841325, PP069382
Bradybaenidae: Helicostylinae	Camaenidae: Helicostylinae	<i>Chloraea intorta</i> (Sowerby, 1840) ^a	Bohol Island, Philippines	S. Chiba	AY841344, PP069336
Bradybaenidae: Helicostylinae	Camaenidae: Helicostylinae	<i>Helicostyla lignaria</i> (Pfeiffer, 1842) ^a	Bohol Island, Philippines	S. Chiba	AY841342 & AY841343, PP069351 ^c
Helminthoglyptidae: Cepolinae	Cepolidae: Cepolinae	<i>Hemitrochus streator</i> (Pilsbry, 1889) [= <i>Cepolis streator</i>] ^a	Grand Cayman	S. Chiba	AY841346, PP069334
Helicidae: Helicigoninae	Helicidae: Ariantinae	<i>Arianta arbustorum</i> (L., 1758) ^a	Deepdale, Derbyshire, UK	C. Wade	AY014136, PP069327
Helicidae: Helicigoninae	Helicidae: Ariantinae	<i>Helicigona lapicida</i> (L., 1758) ^a	Deepdale, Derbyshire, UK	C. Wade	AY014137, PP069352
Helicidae: Helicigoninae	Helicidae: Ariantinae	<i>Isognomostoma isognomostomos</i> (Schröter, 1784) ^b	Polana Mountain, Slo- vakia	J. Grego	PP069357
Helicidae: Helicinae	Helicidae: Helicinae	<i>Allognathus minoricensis</i> (Mittre, 1842) ^b	Lluc, Majorca	C. Wade & B. Wade	PP069325
Helicidae: Helicinae	Helicidae: Helicinae	<i>Cantareus apertus</i> (Born, 1778) ^a	Sicily	A. Davison	AY014129, PP069331
Helicidae: Helicinae	Helicidae: Helicinae	<i>Caucasotachea vindobonensis</i> (C. Pfeiffer, 1828) [= <i>Cepaea vindobonensis</i>] ^b	Katlanovo, North Mace- donia	E. Stojoska & J. Grego	PP069342
Helicidae: Helicinae	Helicidae: Helicinae	<i>Cepaea hortensis</i> (Müller, 1774) ^a	Marlborough Downs, Wiltshire, UK	A. Davison	AY014131, PP069335
Helicidae: Helicinae	Helicidae: Helicinae	<i>Cepaea nemoralis</i> (L., 1758) ^a	Marlborough Downs, Wiltshire, UK	A. Davison	AY014130, PP069338
Helicidae: Helicinae	Helicidae: Helicinae	<i>Cornu aspersum</i> (Müller, 1774) [= <i>Helix aspersa</i>] ^a	Kettering, Northampton- shire, UK	C. Wade	AY014128, PP069353
Helicidae: Helicinae	Helicidae: Helicinae	<i>Eobania vermiculata</i> (Müller, 1774) ^b	Majorca, Spain	Unkown	PP069343
Helicidae: Helicinae	Helicidae: Helicinae	<i>Eremina desertorum</i> (Forskål, 1775) ^a	Unknown	Unknown	AY841335, PP069344
Helicidae: Helicinae	Helicidae: Helicinae	<i>Helix lucorum</i> L., 1758 ^a	Unknown	Unknown	AY841334, PP069319
Helicidae: Helicinae	Helicidae: Helicinae	<i>Helix lutescens</i> Rossmässler, 1837 ^b	Plešivec, Slovakia	J. Grego	PP069354
Helicidae: Helicinae	Helicidae: Helicinae	<i>Helix pomatia</i> L., 1758 ^a	Pulpit Down, Bucking- hamshire, UK	P. Mordan	AY841333, PP069355
Helicidae: Helicinae	Helicidae: Helicinae	<i>Macularia sylvatica</i> (Draparnaud, 1801) [= <i>Cepaea sylvatica</i>] ^b	Switzerland	Unknown	PP069341
Helicidae: Helicinae	Helicidae: Helicinae	<i>Otala lactea</i> (Müller, 1774) ^a	Unknown	Unknown	AY841336, PP069320
Helicidae: Helicinae	Helicidae: Helicinae	<i>Theba pisana</i> (Müller, 1774) ^a	Sicily	A. Davison	AY014134 & AY014135, PP069383
Helicidae: Helicinae	Helicidae: Murellinae	<i>Marmorana scabriuscula</i> (Deshayes, 1830) ^a	Sicily	A. Davison	AY014132 & AY014133, PP069362 ^c

Table 1. Continued

Vaught family/ subfamily	MolluscaBase family/ subfamily	Species	Collection/location	Collector	GenBank accession
Helicidae: Helicodontinae	Helicodontidae: Helicodontinae	<i>Helicodonta obvoluta</i> (Müller, 1774) ^b	West Wood, Winchester, Hampshire, UK	J. S. Gallichan, A. H. Wood & J. Chatfield	PP069349
Hygromiidae: Helicellidae?	Geomitridae: Geomitrinae	<i>Cochlicella acuta</i> (Müller, 1774) ^a	Porthcurnick, Cornwall, UK	E. Bailes	AY014126, PP069339
Hygromiidae: Trichiinae	Geomitridae: Geomitrinae	<i>Ponentina subvirescens</i> (Bellamy, 1839) ^b	Maritime turf on ser- pentine headland, Kynance, Cornwall, UK	G. A. Holyoak	PP069376
Hygromiidae: Helicellidae?	Geomitridae: Helicellinae	<i>Cernuella virgata</i> (Da Costa, 1778) ^a	Porthcurnick, Cornwall, UK	E. Bailes	AY014127, PP069333
Hygromiidae: Hygromiinae	Hygromiidae: Hygromiinae	<i>Monachoides vicinus</i> (Rossmässler, 1842) ^b	Slovakia	J. Steffek	PP069366
Hygromiidae: Hygromiinae	Hygromiidae: Hygromiinae	<i>Perforatella bidentata</i> (Gmelin, 1791) ^b	Bank of Lupcica river, Starohorske Vrchy Mountain, Slovakia	Unknown	PP069373
Hygromiidae: Hygromiinae	Hygromiidae: Trochulininae	<i>Euomphalia strigella</i> (Draparnaud, 1801) ^b	Slovakia	Unkown	PP069346
Hygromiidae: Helicellidae?	Hygromiidae: Trochulininae	<i>Monacha cantiana</i> (Montagu, 1803) ^a	Pulpit Down, Bucking- hamshire, UK	P. Mordan	AY841331 & AY841332, PP069365 ^c
Hygromiidae: Trichiinae	Hygromiidae: Trochulininae	<i>Trochulus striolatus</i> (Pfeiffer, 1828) [= <i>Trichia striolata</i>] ^a	Deepdale, Derbyshire, UK	C. Wade	MN022675, PP069386
Camaenidae: Camaeninae	Pleurodontidae: Lucerninae	<i>Thelidomus aspera</i> (Férussac, 1821) ^a	Windsor, Jamaica	S. Chiba	AY841321, PP069384
Polygyridae: Polygyrinae	Polygyridae: Polygyrinae	<i>Polygyra troostiana</i> I. Lea, 1838 ^b	Cedars of Lebanon State Park, Wilson County, TN, USA	J. Slapcinsky & B. Coles	PP069377
Polygyridae: Ashmunelinae	Polygyridae: Polygyrinae	<i>Triodopsis alleni</i> (Wetherby, 1883) ^a	Williams Creek, IA, USA	R. Cameron	AY841316, PP069385
Polygyridae: Ashmunelinae	Polygyridae: Triodopsinae	<i>Allogona townsendiana</i> (I. Lea, 1838) ^b	Decker Creek at Matlock, Brady Road, Mason County, WA, USA	D. Taylor	PP069324
Polygyridae: Polygyrinae	Polygyridae: Triodopsinae	<i>Inflectarius rugeli</i> (Shuttleworth, 1852) ^b	Polk County, TN, USA	J. Slapcinsky & B. Coles	PP069356
Polygyridae: Polygyrinae	Polygyridae: Triodopsinae	<i>Mesodon thyroides</i> (Say, 1816) ^a	York Co. PA, USA	F. Thompson	AY841315, PP069364
Polygyridae: Ashmunelinae	Polygyridae: Triodopsinae	<i>Vespericola columbiana</i> (Lea, 1838) ^a	Eugene, OR, USA	D. Taylor	AY014120, PP069388
Helminthoglyptidae: Helminthoglyptinae	Xanthonychidae (Helminthoglyptinae): Helminthoglyptinae	<i>Helminthoglypta diaboloensis</i> (J. G. Cooper, 1869) ^b	Sonoma Mountain, CA, USA	T. Lawson	PP069350
Helminthoglyptidae: Helminthoglyptinae	Xanthonychidae: Monadeniinae	<i>Monadenia fidelis</i> (Gray, 1834) ^a	Oregon, USA	D. Taylor	MN022677, PP069368
Camaenidae: Camaeninae	Sagdidae: Polydontinae	<i>Hispaniolana crispata</i> (Férussac, 1821) [= <i>Polydotes undulata</i>] ^a	Dominican Republic	G. Seal	AY014121, PP069375
Sagdidae: Sagdinae	Sagdoidea: Sagdinae	<i>Sagda</i> sp. ^a	Windsor, Jamaica	S. Chiba	AY841347, PP069380
Camaenidae: Camaeninae	Sagdoidea: Zachrysiidae	<i>Zachrysia auricoma</i> (Férussac, 1821) ^a	Nr. Dorado, Puerto Rico	A. Davison	AY841326, PP069390
Camaenidae: Camaeninae	Solaropsidae: Caracolinae	<i>Caracolus marginellus</i> (Gmelin, 1791) ^b	Unknown	G. M. Barker	PP069330 ^c

Table 1. Continued

Vaught family/ subfamily	MolluscaBase family/ subfamily	Species	Collection/location	Collector	GenBank accession
Camaenidae: Camaeninae	Solaropsidae: Caracolinae	<i>Caracolus carocollis</i> (L., 1758) ^b	Yunque, Puerto Rico	A. Davison	PP069374
Outgroup: Haplotrematoidea: Haplotrematidae		<i>Haplotrema vancouverense</i> (Lea, 1839) ^b	Eugene, OR, USA	D. Taylor	AY014090, PP069348

^aSpecimens published by Wade *et al.* (2007) for an ~1460 bp fragment of the 5.8S–ITS2–LSU rRNA (using primers LSU 1–5) and resequenced here for an ~4000 bp fragment of the 5.8S–ITS2–LSU rRNA (using primers LSU 1–12).

^bEntirely new specimens sequenced here for the ~4000 bp fragment of the 5.8S–ITS2–LSU rRNA (using the LSU 1–12 primers).

^cGenBank accession numbers for which the ITS2 fragment is incomplete.

Moreover, some new species were described without considering previous literature, as noted by Wu and Qi (2009) and Zhang (2019), with the species range and morphological characters of the type material not being considered adequately. *Platypetanus* is treated as a synonym of *Pseudiberus* based only on whorl numbers and on shell and umbilicus measurements (Wu and Qi 2009). Moreover, the type species of *Pseudiberus* was recorded in the Taihang Mountains (Zhang *et al.* 2021a), whereas the type species of *Platypetanus* (*Platypetanus innominatus*) was recorded from near Kin-cha Kiang (= Jinsha Jiang) (Heude 1885); the geographical distribution of these taxa is completely different, and their distribution ranges do not overlap. Even some morphological characters can be distinguished, e.g. inner thickness near the aperture (Heude 1885, Wu and Qi 2009, Zhang *et al.* 2021a).

In this study, we: (i) reconstruct a phylogenetic tree based on the large subunit (LSU) ribosomal RNA (rRNA) gene region using Bayesian inference (BI) and maximum likelihood (ML) methods; (ii) examine the taxonomic system of the Helicoidea proposed by Bouchet *et al.* (2017); and (iii) describe new taxa from China based on morphological characters and the molecular phylogeny.

MATERIALS AND METHODS

Biological material

The specimens used in this study are listed in Table 1.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from foot muscle tissue using the CTAB method (Goodacre and Wade 2001). Approximately 4000 bp of the LSU rRNA gene was amplified using the polymerase chain reaction (PCR). The LSU rRNA, embracing part of 5.8S and ITS2, was amplified by nested PCR using the primers LSU-1ii and LSU-12 to produce primary PCR products, then using the primary PCR products as the template for secondary PCR. Secondary PCR was used to amplify six fragments (A–F) using the primers LSU-1iii and LSU-3iii (fragment A), LSU-2ii and LSU-5ii (fragment B), LSU-4ii and LSU-7 (fragment C), LSU-6i and LSU-9i (fragment D), LSU-8ii and LSU-11ii (fragment E), and LSU-10i and LSU-12i (fragment F) (Wade and Mordan 2000, Fontanilla *et al.* 2017). PCR amplifications for both primary and secondary PCRs were performed using Promega GoTaq[®] G2 Master Mix buffer (1 U TAQ, 0.2 μM primers, 200

μM dNTP, and 1.5 mM MgCl₂), with 1 μL of DNA template added to 24 μL of 1× Master Mix buffer. The cycling conditions of the primary PCR were as follows: 96°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 3 min, then a final extension step at 72°C for 5 min. The secondary PCR cycle conditions were as follows: 96°C for 2 min, followed by 35 cycles of 94°C for 30 s, 45°C for 1 min, and 72°C for 2 min, then a final extension step at 72°C for 5 min. The PCR products were purified using the Qiagen gel extraction kit, and sense and anti-sense sequencing was performed by Macrogen Inc.

Sequence analysis

DNA sequence trace data for the 12 sequence fragments were assembled using DNASTAR SEQMAN v.7.1.0 (Swindell and Plasterer 1997) and the STADEN package (Staden *et al.* 2000). After assembly, sequences were aligned by MAFFT v.7.505 (Nakamura *et al.* 2018), with the alignment improved manually in the Genetic Data Environment (GDE) package (Smith *et al.* 1994). Ambiguously aligned sequence regions were removed from the alignment before phylogenetic analysis using GBLOCKS v.0.91b (Castresana 2000) (maximum number of contiguous non-conserved positions of five, and minimum length of a block of three), then checked again in GDE. Three thousand, four hundred and twelve sites were deemed to be aligned unambiguously and were used for subsequent tree building.

Phylogenetic trees were constructed using BI and ML methods. The general time reversible (GTR) model incorporating gamma distribution (+Γ) was chosen as the best model by MODELTEST-NG v.0.1.7 (Darriba *et al.* 2019) based on the Bayesian information criterion. Bayesian inference phylogenies were generated using MRBAYES v.3.2.7 (Ronquist *et al.* 2012), with two runs for 5 000 000 generations, sampling every 100 generations, and a temperature of 0.05. The final tree and posterior probabilities (PPs) were determined based on the last 50% of trees (burn-in = 0.5) and majority rule consensus. The split frequencies before burn-in were <0.01. Maximum likelihood phylogenetic trees were generated using RAXML-NG v.1.1.0 (Kozlov *et al.* 2019), using a heuristic search strategy with 10 random plus 10 parsimony start trees and with subtree pruning and regrafting (SPR) branch swapping. Bootstrap analysis [transfer bootstrap expectation (TBE); Lemoine *et al.* 2018] was undertaken for the ML tree with 1000 bootstrap replicates and shown as a percentage. Here, ML = 100%, PP = 1.00 is considered as full support, ML > 90%, PP > .9 is considered

as strong support, and ML > 70%, PP > .8 is considered good support; anything lower is considered as weak support.

GenBank accession numbers

GenBank accession numbers for the sequences generated in this study are recorded in [Table 1](#).

RESULTS

Molecular phylogenetic analyses

The Bayesian tree of the Helicoidei is shown in [Figure 1](#). Both the Helicoidea (BI PP = 1.00 and 97% ML bootstrap) and the Sagdoidea (PP = 1.00, 100% ML) form strongly supported monophyletic groups in the tree, and the Helicoidea and Sagdoidea cluster as sister groups with full support (PP = 1.00, 100% ML). The Bayesian and ML phylogenetic trees are highly consistent.

Sagdoidea

The Sagdoidea include Solaropsidae, Sagdidae, and Zachrysiidae. The Solaropsidae, represented by *Caracolus marginella* (Gmelin, 1791) and *Caracolus carocolla* (L., 1758), form a fully supported monophyletic group in the tree (PP = 1.00, 100% ML). Likewise, the Sagdidae, represented by two species, *Hispaniolana crispata* (Férussac, 1821) [= *Polydontes undulata*] and *Sagda* sp., also form a fully supported monophyletic group (PP = 1.00, 100% ML). The Zachrysiidae contain only a single species, *Zachrysia auricoma* (Férussac, 1821), in our phylogenetic tree. Sagdidae and Zachrysiidae are sister groups with good support (PP = .99, 86% ML), and Solaropsidae is the sister to the clade comprising Sagdidae + Zachrysiidae (PP = 1.00, 100% ML).

Helicoidea

The family Cepolidae is sister to all other Helicoidea with strong support (PP = 1.00, 97% ML). It is represented by a single taxon, *Hemitrochus streator* (Pilsbry, 1889) [= *Cepolis streator*], in our tree. The remaining Helicoidea can be divided into two sister groups. The first group comprises Hygromiidae s.l., Xanthonychidae, Pleurodontidae, Helicodontidae, and Helicidae with strong support (PP = .94, 91% ML). It is, in turn, divided into two sister groups, the Helicidae with strong support (PP = 1.00, 99% ML) and the Hygromiidae s.l., Xanthonychidae, Pleurodontidae, and Helicodontidae with good support (PP = .94, 75% ML). The second group comprises the Camaenidae + Bradybaenidae + Polygyridae and is strongly supported (PP = 1.00, 96% ML).

Helicidae

The Helicidae consist of a sister-group relationship between the Helicinae and Murellinae + Ariantinae. The Helicinae are strongly supported (PP = .90, 94% ML), but the grouping of Murellinae and Ariantinae has weak support (PP = .66, 65% ML). The Ariantinae are weakly supported in our analyses (PP = .55, 62% ML), and the Murellinae contain only a single species in our phylogenetic tree.

The subfamily Helicinae forms a polytomy, comprising three lineages in the phylogenetic tree. The first lineage exhibits a sister-group relationship between *Helix* spp. and *Caucasotachea*

vindobonensis (C. Pfeiffer, 1828) [= *Cepaea vindobonensis*], which has only weak support in BI (PP = .51); ML does not support this topology ([Supporting Information, Fig. S1](#)). The second lineage comprises a single species, *Eremina desertorum* (Forskål, 1775). The final lineage is composed of all remaining Helicinae species and is strongly supported (PP = 1.00, 98% ML). In this lineage, (*Macularia sylvatica* (Draparnaud, 1801) [= *Cepaea sylvatica*] + *Allognathus minoricensis* (Mittre, 1842) + *Cepaea* spp.) is sister to (*Theba pisana* (Müller, 1774) + *Cornu aspersum* (Müller, 1774) [= *Helix aspersa*] + *Cantareus apertus* (Born, 1778) + *Eobania vermiculata* (Müller, 1774) + *Otala lactea* (Müller, 1774)) (PP = 1.00, 98% ML). *Allognathus minoricensis* (Mittre, 1842) is sister to *Cepaea* spp. with full support (PP = 1.00, 100% ML), and *Macularia* is the sister group of *Allognathus* and *Cepaea* spp. with strong support (PP = 1.00, 91% ML). *Theba pisana* is the sister group of *Cornu aspersum* [= *Helix aspersa*] + *Cantareus apertus* + *Eobania vermiculata* (Müller, 1774) + *Otala lactea* with strong support (PP = .96, 97% ML). *Cornu* is the sister to *Cantareus* with strong support (PP = 1.00, 99% ML). *Eobania* is sister to *Otala* with weak support in BI (PP = .59) but strong support in ML (79% ML). *Cornu* + *Cantareus* is the sister group of *Eobania* + *Otala* with strong support (PP = 1.00, 97% ML).

Marmorana scabriuscula (Deshayes, 1830) is the only representative of Murellinae in our phylogenetic tree. *Arianta arbustorum* (L., 1758) is sister to the other Ariantinae (PP = .66, 65% ML), and *Isognomostoma isognomostomos* (Schröter, 1784) and *Helicigona lapicida* (L., 1758) are sister groups with strong support in BI (PP = .90) but weak support in ML (55% ML).

Xanthonychidae

The Xanthonychidae contain *Monadenia fidelis* (Gray, 1834) and *Helminthoglypta diabloensis* (Cooper, 1869), forming a monophyletic group with good support in BI (PP = .80) but weak support in ML (54%).

Pleurodontidae

Thelidomus aspera (Férussac, 1821) is our only representative of Pleurodontidae, which is sister to Xanthonychidae with good support in BI (PP = .89) but weak support in ML (62%).

Helicodontidae

The Helicodontidae are sister to Hygromiidae s.l. + Xanthonychidae and Pleurodontidae with good support (PP = .94, 75% ML) and have only one species, *Helicodonta obvoluta* (Müller, 1774).

Hygromiidae s.l.

The Hygromiidae s.l. form a monophyletic group with good support (PP = .99, 78%). Hygromiidae s.l. are sister to Xanthonychidae and Pleurodontidae in the tree, with good support (PP = .95, 78% ML). Among Hygromiidae s.l., the Trochulininae (Hygromiidae) (PP = 1.00, 99% ML) are sister to other Hygromiidae s.l. and contain *Trochulus striolatus* (Pfeiffer, 1828) [= *Trichia striolata*], *Monacha cantiana* (Montagu, 1803), and *Euomphalia strigella* (Draparnaud, 1801). Geomitridae (PP = 1.00, 98% ML) and Hygromiinae (Hygromiidae) (PP = 1.00, 100% ML) are sister groups with good support

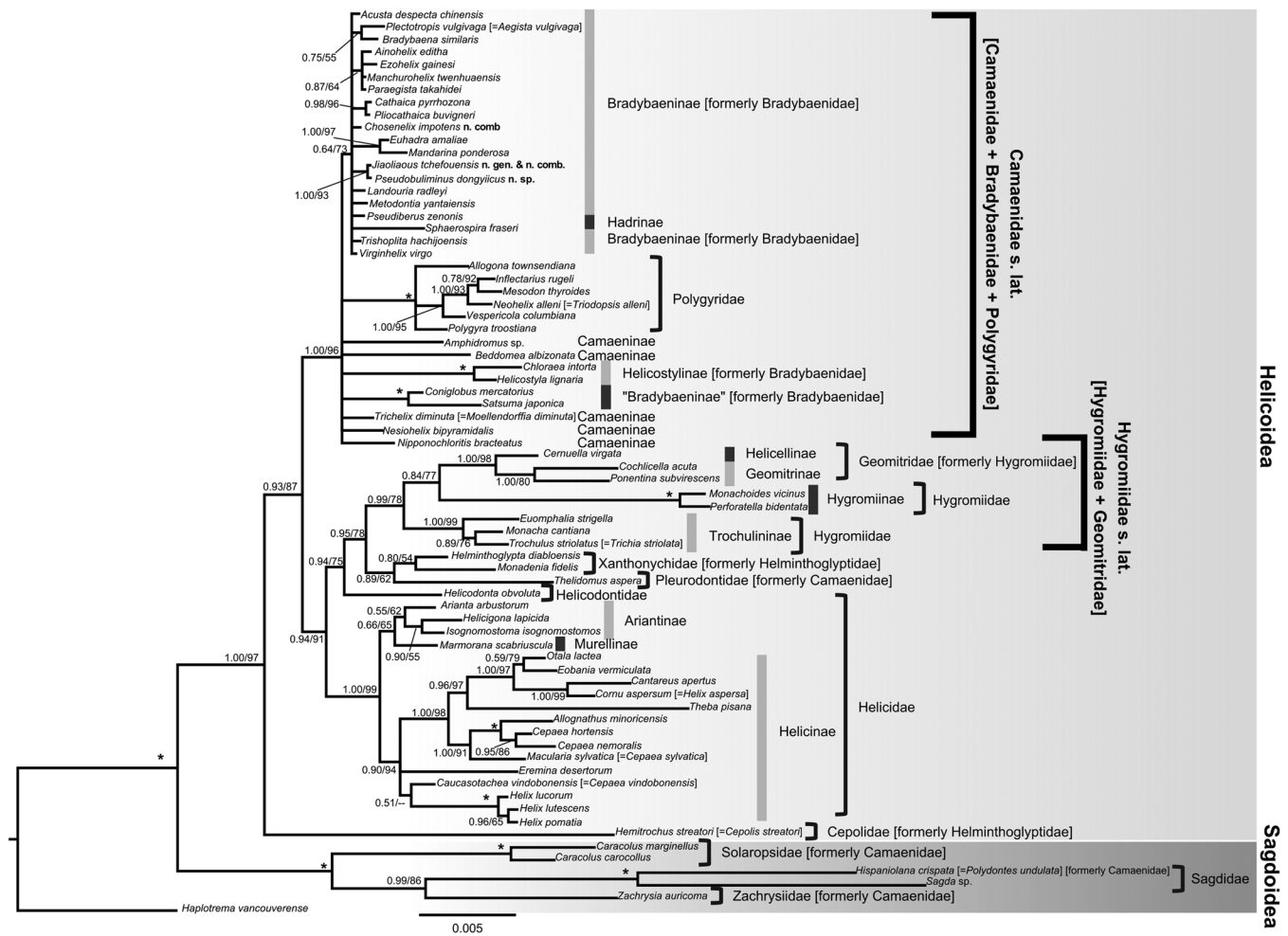


Figure 1. Bayesian inference phylogenetic tree of the Helicoidei based on LSU rDNA sequences. The tree is rooted on the outgroup *Haplotrema vancouverense*. Bayesian posterior probabilities and ML bootstrap values are given in the following order: BI/ML. Star symbol denotes full support (PP = 1.00, 100% ML). Details of specimens can be found in Table 1. Family and subfamily classifications are shown on the tree (with previous family classifications shown in square brackets). The scale bar corresponds to five nucleotide substitutions for every 1000 nucleotides.

(PP = .84, 77% ML). The Hygromiinae contain *Monachoides vicinus* (Rossmässler, 1842) and *Perforatella bidentata* (Gmelin, 1791). For Geomitridae, Geomitrinae (PP = 1.00, 80% ML) and Helicellinae are sister groups, and *Ponentina subvirescens* (Bellamy, 1839) and *Cochlicella acuta* (Müller, 1774) in Geomitrinae are sister taxa. The Helicellinae contain only one species, *Cernuella virgata*.

Camaenidae s.l.

The clade of Camaenidae + Bradybaenidae + Polygyridae is strongly supported (PP = 1.00, 96% ML). There is a polytomy at the base of the clade, which comprises: (i) Bradybaeninae (excluding *Satsuma* and *Coniglobus*), along with Hadrinae; (ii) a separate branch consisting of *Satsuma* and *Coniglobus*; (iii) Helicostyliinae; (iv) Polygyridae; and (v) numerous individual lineages of Camaeninae genera, such as *Nipponochloritis*, *Nesiohelix*, *Trichelix*, *Beddomea*, and *Amphidromus*. The main Bradybaeninae clade including the Hadrinae (but excluding *Satsuma* and *Coniglobus*) is weakly supported in BI (PP = .64) but well supported in the ML analysis (73% ML). Within this, the following sister groups are recognized: *Jiaoliaous tchefouensis* (Crosse & Debeaux, 1863) and *Pseudobuliminus dongyiicus*

Zhang, n. sp. (PP = 1.00, 93% ML); *Euhadra* and *Mandarina* (PP = 1.00, 97% ML); *Cathaica pyrrhizona* (Philippi, 1845) and *Pliocathaica buvigneri* (Deshayes, 1873) (PP = .98, 96% ML); and *Plectotropis* and *Bradybaena* (PP = .75, 55% ML). *Ainohelix*, *Ezohelix*, *Manchurohelix*, and *Paraegista* from North East Asia also cluster together in the tree (PP = .87, 64% ML). *Satsuma* and its previous subgenus, *Coniglobus*, cluster together, forming a monophyletic clade with full support (PP = 1.00, 100% ML), but fall separately from the main Bradybaeninae clade. Helicostyliinae are a monophyletic group with full support (PP = 1.00, 100% ML). Polygyridae are also fully supported (PP = 1.00, 100% ML).

NOMENCLATURE

Helicoidea Rafinesque, 1815

Bradybaenidae Pilsbry, 1898

Bradybaeninae Pilsbry, 1898

Jiaoliaous Zhang gen. nov.

[urn:lsid:zoobank.org:act:83C51892-AFB9-4CDB-B2BF-81E824BAD258]

Type species: Helix tchefouensis Crosse & Debeaux, 1863, original designation.

Jiaoliaous tchefouensis (Crosse & Debeaux, 1863) gen. nov., comb. nov.

The taxonomic description of *Jiaoliaous tchefouensis* can be found in the [Supporting Information](#), with photographs of *Jiaoliaous tchefouensis* in the [Supporting Information](#) (Fig. S2).

Pseudobuliminus Gredler, 1886

Type species: Helix pseudobuliminus Heude, 1882, absolute tautonymy.

Pseudobuliminus dongyiicus Zhang sp. nov.

[urn:lsid:zoobank.org:act:D44A4F43-6A43-4C3B-95F8-74C4221B5ABD]

The taxonomic description of *Pseudobuliminus dongyiicus* can be found in the [Supporting Information](#), with photographs of *Pseudobuliminus dongyiicus* in the [Supporting Information](#) (Fig. S3).

DISCUSSION

Helicoidea

Camaenidae s.l.

The Camaenidae, Bradybaenidae, and Polygyridae cluster in our phylogeny with strong support to form the Camaenidae *s.l.* (Fig. 1; Supporting Information Figs. S1 and S4). Relationships within this group are not well resolved, and the Polygyridae form one of the multifurcating branches alongside other camaenid and bradybaenid taxa in a polytomy at the base of the clade. [Wade et al. \(2007\)](#) showed that the Camaenidae from Asia and Australia were a separate group from the American taxa, with these American camaenids subsequently moved to the Sagdoidea and Pleurodontidae by [Sei et al. \(2017\)](#); an arrangement adopted by [Bouchet et al. \(2017\)](#). The remaining Asian and Australian camaenid taxa clustered with the Bradybaenidae in the tree of [Wade et al. \(2007\)](#); subsequently, [Gittenberger et al. \(2012\)](#) and [Bouchet et al. \(2017\)](#) synonymized the Bradybaenidae with the Camaenidae to form the Camaenidae *s.l.* (Fig. 1). We followed the subfamily classification of [Bouchet et al. \(2017\)](#), including four subfamilies, the Camaeninae [Pilsbry, 1895](#), Bradybaeninae [Pilsbry, 1934](#) (1898), Hadrinae [Iredale, 1937](#), and Helicostylinae [Ihering, 1909](#) (these subfamily relationships are consistent with those established in early taxonomic studies; [Pilsbry 1895, 1934](#), [Ihering 1909](#), [Iredale 1937](#)). In our phylogenetic tree, Hadrinae cluster with the Bradybaeninae (excluding *Satsuma* and *Coniglobus*), with *Satsuma* + *Coniglobus*, the Helicostylinae, the Polygyridae, and several other individual Camaeninae genera forming part of a polytomy at the base of the Camaenidae *s.l.*

Our phylogenetic tree differs in some respects from the mitochondrial phylogenies of [Minton et al. \(2016\)](#), [Zhang et al. \(2021b\)](#), and [Zhao et al. \(2023\)](#), from which there are three different hypotheses regarding the relationships within the Camaenidae *s.l.* The first hypothesis, based on the ML phylogenetic trees of [Minton et al. \(2016\)](#) and [Zhang et al. \(2021b\)](#), places the Polygyridae as sister to the Helicidae + Bradybaenidae + Camaenidae, and additionally the Geomitridae for [Zhang](#)

[et al. \(2021b\)](#), while the Bradybaenidae are sister to the Camaenidae with strong support. The second hypothesis, based on the ML Gene-Order (MLGO) tree of [Minton et al. \(2016\)](#), shows that the Camaeninae, Bradybaeninae, and Polygyridae cluster together but with low support, with Bradybaenidae sister to Polygyridae instead of Camaenidae. The final hypothesis, based on the phylogeny of [Zhao et al. \(2023\)](#), places the Polygyridae as sister to the Camaenidae + Bradybaenidae with strong support, and the Camaenidae are sister to the Bradybaenidae with strong support. Our results for the Camaenidae *s.l.* are similar to those of [Zhao et al. \(2023\)](#), but the internal topology differs.

The subfamily groupings in our phylogenetic tree are consistent with currently established taxonomic classifications ([Bouchet et al. 2017](#)), but they are inconsistent with the phylogeny based on genital characters as proposed by [Wu \(2004\)](#). In Wu's phylogeny, the Bradybaeninae are considered paraphyletic and cluster with the Helicostylinae under successive weighting, implied weighting, and extended implied weighting ([Wu 2004](#); [Supporting Information, Fig. S5B, C](#)), although support is weak, there is no apomorphy, and only a single Camaeninae member was represented ([Supporting Information, Fig. S5](#)). Under equal weighting, the grouping of subfamilies was also unresolved, with weak support for branches and with no apomorphy. Bradybaeninae formed a polyphyletic cluster with the single Camaeninae member, *Camaena*. Helicostylinae remained monophyletic ([Supporting Information, Fig. S5A](#)). Examination of our and [Wu's \(2004\)](#) parsimony phylogenetic techniques indicate that these differences are likely to stem from inaccuracies in the morphological character matrix ([Goloboff and Morales 2023](#)).

Based on both morphology (without the dart apparatus) and the molecular phylogenetic results of [Wade et al. \(2006\)](#) and [Wade et al. \(2007\)](#), it has already been noted in earlier literature, such as by [Nordsieck \(2017\)](#), that *Satsuma* is unique in comparison to other bradybaenid species from East Asia. The Bayesian tree of [Sei et al. \(2017\)](#) suggested that *Satsuma* is sister to other Camaenidae (excluding *Satsuma* in the study by [Sei et al. 2017](#)), Bradybaenidae, and Polygyridae. In this study, *Satsuma s.l.* (*Satsuma* including its subgenus, *Coniglobus*) is a monophyletic group distinguished from the lineage of Bradybaeninae and Hadrinae. Our conclusion is that *Satsuma* and its previous subgenera (*Coniglobus*, *Luchuhadra*, and *Satsuma s.s.*) should be recognized as a separate subfamily. Its members are endemic to Taiwan Island, the Ryukyu Islands, Shikoku Island, and Honshu Island ([Hoso et al. 2010](#)).

Although the Helicostylinae appear as a monophyletic group in our phylogenetic tree, the phylogenetic relationships within the Helicostylinae, as presented by [Batomaque et al. \(2019\)](#) based on *COI*, exhibit a greater level of complexity, consisting of three polytomic lineages. In the first lineage, *Chloraea* (Helicostylinae) clusters with *Aegista* spp. (Bradybaeninae). The second lineage comprises only Helicostylinae, and the final lineage, a subset of Helicostylinae (*Phoenicobius* and *Chysallis*), clusters with *Nesiohelix* (Camaeninae) and *Satsuma* from Taiwan. Our phylogeny contradicts the topology of [Batomaque et al. \(2019\)](#), in that *Aegista* is shown to be sister to *Bradybaena*, unequivocally falling within the Bradybaeninae. This discrepancy suggests a need for further examination based on 28S of the monophyly of Helicostylinae, with an emphasis on increased sampling.

In our phylogenetic tree, Bradybaeninae and Hadrinae, represented in our phylogeny by a single species, *Sphaerospira fraseri* from East Australia, cluster together to form a monophyletic group. The Bradybaeninae species within this lineage are mostly distributed in East Asia, with only a single genus, *Landouria* Godwin-Austen (1918), ranging across the southern Himalaya. *Landouria radleyi* has a disjunct occurrence in the Central Highlands of Sri Lanka. However, the placement of eastern species in *Landouria* has been challenged based on morphological characters (Páll-Gergely *et al.* 2013).

The Hadrinae group with the Bradybaeninae in both BI and ML analyses. However, we note that *Sphaerospira fraseri* falls on a relatively long branch in comparison to other Bradybaeninae within the group, and there is therefore a possibility that this placement might be attributable to long branch attraction. Our equal-weighting parsimony phylogeny (Supporting Information, Fig. S4) weakly supports the monophyly of Bradybaeninae based on jackknifing support and suggests Hadrinae as a distinct polytomy branch of the Camaenidae *s.l.* Our parsimony tree is similar to the phylogeny reconstructed by Sei *et al.* (2017), in which Bradybaeninae are sister to Polygyridae, and Hadrinae are sister to Polygyridae and Bradybaeninae. Considering the geographical distribution, we encounter a conflict with our phylogenetic results. Wallace's line remains a relevant barrier for terrestrial Mollusca (Hausdorf, 2019), and Bradybaeninae from East Asia and Hadrinae from Australia are separated by Wallace's line. According to Razkin *et al.* (2015) and Sei *et al.* (2017), the Bradybaenidae separated from the common ancestor of Bradybaenidae + *Satsuma* ~47.49 Mya. This time point should be earlier than separation of Bradybaeninae and Hadrinae based on our topology. However, at that time, Asia and Australia were no longer connected, conflicting with the hypothesis that the Bradybaeninae are close relatives of the Hadrinae.

As a whole, the Camaenidae *s.l.*, including the Camaenidae, Bradybaenidae, and Polygyridae, are distributed in different regions of the world: East Asia including Taiwan Island, the Ryuku Islands and Shikoku Island (Bradybaeninae), Taiwan Island, Ryukyu Islands, Shikoku Island (subfamily including *Satsuma* and its previous subgenera), Australia (Hadrinae), Southeast and South Asia (Camaeninae), Philippines (Helicostylinae), and North America (Polygyridae). Preliminarily, our results match the original distribution definitions of these Bradybaenidae, Camaenidae, and Polygyridae subfamilies, with the exception of the Camaeninae (Pilsbry 1895, 1934, Ihering 1909, Iredale 1937), which was not defined by a specific region by previous authors, but in this study is mainly from Southeastern Asia. When discussing the internal grouping of Camaenidae *s.l.*, the lack of resolution has to be addressed. One plausible explanation is that, following their colonization of a new geographical area and range of habitats, they underwent a phase of rapid diversification.

Hygromiidae s.l.

Our phylogenetic tree resolves the Hygromiidae *s.l.* as defined by Bouchet and Rocroi (2005) and includes Geomitridae and Hygromiinae as monophyletic subfamily groups. Bouchet *et al.* (2017) subsequently accepted the phylogenetic results of Razkin *et al.* (2015), which split the Hygromiidae into three families, the Canariellidae, Geomitridae, and Hygromiidae. However, the Hygromiidae defined by Razkin *et al.* (2015) are

not monophyletic in our phylogenetic trees, and the topologies in BI and ML are consistent, with good support. This can be resolved by recognizing what is a more stable level of family hierarchy in the broader context of the tree and synonymizing the Geomitridae with the Hygromiidae and recognizing the Helicellinae, Geomitridae, Hygromiinae, and Trochulininae within the Hygromiidae.

Razkin *et al.* (2015) and Neiber *et al.* (2017) both adopted a multi-gene phylogenetic approach based on mitochondrial sequence data for the Hygromiidae *s.l.* Both studies used the mitochondrial 16S rRNA gene, and Neiber *et al.* (2017) also incorporated the mitochondrial *CO1* section. Notably, previous research by Mardulyn and Whitfield (1999) found that 16S and *CO1* phylogenies at the genus level exhibited a lack of signal and were prone to noise, although we note that Fontanilla *et al.* (2017) successfully used the mitochondrial *CO1* gene to look at family-level relationships within the Achatinoidea by restricting analyses to the more conservative first and second codon positions and excluding the saturated third codon positions. Mengual *et al.* (2008), Zaldivar-Riverón *et al.* (2008), Klopstein *et al.* (2010), and Townsend and Leuenberger (2011) have suggested that the nuclear 28S rRNA gene is a superior marker at ancient time scales when compared with non-conserved genes, such as *CO1*. Nevertheless, it is important to acknowledge that phylogenies based on the 28S marker still exhibit limited resolution in understanding the relationships within Hygromiidae *s.l.* In addition, our parsimony tree (Supporting Information, Fig. S4) treated each subfamily as a single branch within the multifurcating lineage of Helicoidea, with the exception of Hygromiinae, which grouped together with Cepolidae. This observation implies that 28S might not be sufficient to elucidate the relationships among these subfamilies.

Xanthonychidae

Although our phylogenetic tree suggests that the Xanthonychidae are monophyletic, it is important to consider the broader context. Previous phylogenetic studies on the Xanthonychidae based on morphological data, including the study by Cuzzo (1998), have raised doubts about their monophyly. These doubts stem from factors such as geographical distribution and morphological phylogeny. Calcutt *et al.* (2020) have provided support for the hypothesis that the Xanthonychidae are not monophyletic. Their molecular phylogenetic analysis suggested that Xanthonychidae can be divided into four distinct groups. This finding contrasts with the results of Frank Koehler's phylogenomic research as reported by Bouchet *et al.* (2017). The biogeographical distribution also appears to align more with the findings of Calcutt *et al.* (2020). According to Miller and Naranjo-García (1991), the Monadeniinae are primarily distributed in the North Rocky Mountains, Helminthoglyptinae in the Middle Rocky Mountains, Humboldtianinae in the South Rocky Mountains, Xanthonychidae in Central America, and Epiphragmophorinae in the Andes Mountains of South America. It is essential to note that our taxon sampling of Xanthonychidae focuses primarily on Monadeniinae and might not be representative of the entire diversity within Xanthonychidae.

Pleurodontidae

In the study by Wade *et al.* (2007), there are three lineages of American Camaenidae. Two of these lineages are now

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