

The introduced terrestrial slugs *Ambigolimax nyctelius* (Bourguignat, 1861) and *Ambigolimax valentianus* (Férussac, 1821) (Gastropoda: Limacidae) in California, with a discussion of taxonomy, systematics, and discovery by citizen science

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The introduced terrestrial slugs *Ambigolimax nyctelius* (Bourguignat, 1861) and *Ambigolimax valentianus* (Férussac, 1821) (Gastropoda: Limacidae) in California, with a discussion of taxonomy, systematics, and discovery by citizen science

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ABSTRACT

Ambigolimax valentianus and *Ambigolimax nyctelius* are synanthropic terrestrial slugs that have been dispersed widely by human activity. Herein, these species are reported from Los Angeles County, California, as a result of contributions to SLIME (Snails and Slugs Living in Metropolitan Environments), a citizen science project initiated by the Natural History Museum of Los Angeles County and hosted online by iNaturalist. Importantly, collected specimens and specimen data of *A. nyctelius* are the first of this species in California and the first records to document it in North America since 1960. Species identifications of *A. valentianus* and *A. nyctelius*, which are phenotypically similar, were made by examination of specimens' distal genitalia and analysis of their mtDNA barcoding gene, COI (*A. nyctelius*, $n = 18$ and *A. valentianus*, $n = 11$). Slug radulae and jaws were examined and are figured, but were not diagnostic to species. Phylogenetic analyses of COI haplotypes from both *Ambigolimax* species and six other limacids resolve *A. nyctelius* and *A. valentianus* as monophyletic sister taxa. However, morphological and molecular data from taxa not included in this study are needed to substantiate this relationship and inform a revision of at least 10 genera in the family Limacidae. Inconsistencies in the literature regarding the year of Férussac's species description of *A. valentianus* (as *Limax valentianus*) are also discussed and resolved.

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Introduction

Many terrestrial gastropod taxa have been widely dispersed by anthropogenic means and have acclimated or adapted to habitats where they are non-native (Cowie and Robinson 2003; Kappes and Schilthuizen 2014; Mc Donnell and Hahs 2015). Synanthropic slugs are among these 'travelling species' (Robinson 1999) and may be

widespread and invasive in metropolitan and agrarian environments worldwide. As a result, distant locales may have similar terrestrial slug fauna of sympatric non-native species (Robinson 1999; Araya 2015). For example, the native European slugs *Deroceras reticulatum* (Müller, 1774), *Arion hortensis* Férussac, 1819, and *Limacus flavus* (Linnaeus, 1758) are co-occurring and pestiferous 'exotics' in New Zealand (Barker 1999), South Africa (Herbert 2010), and California (Roth and Sadeghian 2006).

Ambigolimax valentianus (Férussac, 1821) is a limacid slug originally native to the Iberian Peninsula that has become a widespread synanthrope (Barker 1979) in greenhouses and anthropogenically altered environments, including in the USA and Canada (Quick 1960; Chichester and Getz 1973; McCracken and Selander 1980; Grimm et al. 2009; von Proschwitz 2009; Rowson et al. 2014b). In 1917, *A. valentianus* was recorded for the first time in North America (as *Limax arborum* Bouchard-Chantereaux, 1837) from an orchid greenhouse in Boulder, Colorado (Cockerell 1917). In 1944, it was reported as abundant and widely distributed in southern California (Gregg 1944), and it continues to be identified in the region (Roth and Sadeghian 2006; Mc Donnell et al. 2009). Notably, *A. valentianus* is often externally indistinguishable from its congener, *Ambigolimax nyctelius* (Bourguignat, 1861), which is also pestiferous and has become established outside of its native range of south-eastern Europe (Barker 1999; Anderson 2005; Welter-Schultes 2012).

In North America, *A. nyctelius* has been reported only once, in 1960, from date palms in Washington, DC (Quick 1960), and has not been identified in subsequent regional inventories of terrestrial gastropods (e.g. Chichester and Getz 1973; Getz et al. 2017). Although some authors list *A. nyctelius* as introduced to the USA (e.g. van Regteren Altena 1966; van Regteren Altena and Smith 1975; Herbert 1997; Borredà and Martínez-Ortí 2017), as far as the authors are aware, there have been no substantiated reports of *A. nyctelius* in North America since 1960, and no documentation of this species as established in California. Neither *A. nyctelius* nor *A. valentianus* has been evaluated for its pest potential by the California Department of Food and Agriculture (CDFA) and few institutionally directed efforts have surveyed the local or California-wide malacofauna, especially the slugs (Mc Donnell et al. 2009).

To improve the inventory of terrestrial gastropods in southern California and especially Los Angeles County, the most populous county in the USA (US Census Bureau 2018), the Natural History Museum of Los Angeles County (NHMLA) began the Snails and slugs Living in Metropolitan Environments (SLIME) project, a citizen or community science initiative (hereafter referred to as citizen science), in 2015. The project's goal is to document the terrestrial malacofauna in Los Angeles County and southern California by specimen collection and species observations contributed by the public as digital photographs to the online biodiversity platform iNaturalist (www.inaturalist.org/projects/slime). SLIME was modelled after another citizen science initiative begun at NHMLA called RASCals (Reptiles and Amphibians of Southern California), which has generated multiple new Los Angeles County records of introduced species (e.g. Pauly et al. 2014, 2015).

In 2016, SLIME contributors posted photos with geo-referenced data of putative *A. nyctelius* slugs to iNaturalist from localities throughout Los Angeles County. Subsequent collection and analysis of these slugs confirmed, for the first time, that *A. nyctelius* was established at multiple sites in the city of Los Angeles and in adjacent cities and suburbs.

The very first record of *A. nyctelius* in California, and the first COI sequence derived from a specimen of this species from North America, is likely that of a single slug collected by R. Mc Donnell at a garden centre in Arcata, Humboldt County, California in 2007. Unfortunately, after this specimen was sequenced for its barcoding gene (COI), it was accidentally discarded by janitorial staff. Thus, as far as the authors are aware, *A. nyctelius* specimens from Los Angeles County deposited in NHMLA's Malacology Collection are the first vouchered specimens of this species from the Los Angeles metropolitan area, and the first representatives of this species as established in North America.

Species identity of *A. nyctelius* and *A. valentianus* was confirmed using an integrative taxonomy approach (Nitz et al. 2010; Padial et al. 2010) that paired independent examination of morphology (external phenotype, phallus, jaw, and radula) with molecular data (COI sequences). Given that limacids are hermaphroditic, all sexually mature slugs have developed distal male genitalia that have been shown to be diagnostic to species (Mc Donnell et al. 2009). Dorsal mantle pattern is inter and intra-specifically variable in *A. nyctelius* and *A. valentianus* and is an unreliable indicator of species identity. Likewise, radular and jaw morphology are usually not diagnostic to species in limacids, but do display intra and interspecific variation that is rarely presented in the literature (Nitz et al. 2009). Therefore, we illustrate distal male genitalia as well as external phenotype, radulae, and jaws of *A. nyctelius* and *A. valentianus* for comparative purposes, and provide barcoding (COI) sequences for individuals of both species to inform taxonomic treatments and phylogenetic analyses.

Barcoding sequences from *A. nyctelius*, *A. valentianus*, and other limacids with publicly available COI data, were analysed for haplotype divergence and used to create a phylogenetic hypothesis for limacids in six genera and eight species using maximum likelihood and Bayesian inference methods. According to Rowson et al. (2014b), *A. nyctelius* and *A. valentianus* are the only species within the genus *Ambigolimax*. This taxonomy is followed here and is discussed, as is the publication year of Férussac's species description of *A. valentianus*.

Taxonomy of *Ambigolimax nyctelius*

Bourguignat described *Ambigolimax nyctelius* in 1861 as *Limax nyctelius* in 'Des limaces algériennes', based on specimens in alcohol collected in Algeria (Bourguignat 1861). In 1887, Pollonera treated *Malacolimax* Malm, 1868 as a subgenus of *Agriolimax*, which included *Agriolimax (Malacolimax) nyctelius* and three other species. This taxonomy was rejected and revised by many authors including Germain (1907), Quick (1960), and van Regteren Altena (1966), all of whom preferred to retain Bourguignat's species in *Limax*. Lupu (1971) was the first to transfer *Limax nyctelius* into the genus *Lehmannia* Heynemann, 1863, and did so as *Lehmannia nyctelius*. Many subsequent references to this species, some of which documented it for the first time in South Africa, Australia, New Zealand, and the UK, refer to it as *Lehmannia nyctelia* (Barker 1979, 1999; Kerney and Cameron 1979; Anderson 2005; Herbert 2010; Benocci et al. 2014). In 2014, Rowson et al. (2014a, 2014b) were the first to publish the species as *Ambigolimax nyctelius*, which has been recently adopted by the WoRMS database (Bank and Neubert 2017; WoRMS Editorial Board 2018).

Taxonomy of *Ambigolimax valentianus*

Ambigolimax valentianus was described in 1821 by Férussac as *Limax valentianus* in 'Tableaux systématiques des animaux mollusques...' based on specimens in alcohol that were collected in Spain (Férussac 1821). In 1887, Pollonera re-described this species as *Agriolimax* (*Ambigolimax*) *valentianus* also based on specimens collected in Spain, citing inadequacies in Férussac's original description (Pollonera 1887). In the same paper, Pollonera (1887) defined *Ambigolimax* as a subgenus based on the slugs' phallus having a globular or bag-like appendix [appendice borsiforme]. This taxonomy was rejected by Simroth (1891) and revised and/or commented on by many authors (e.g. Hesse 1926; Kennard and Woodward 1926; van Regteren Altena 1958; Quick 1960; Waldén 1961; Stears 1974; Grossu and Tesio 1975), all of whom favoured the genus name *Lehmannia* or *Limax* Linnaeus, 1758. Of these taxonomic treatments, Kennard and Woodward (1926) is of particular significance because it classifies *Agriolimax* (*Ambigolimax*) *fulvus* (= *Limax fulvus* Normand, 1852), the only other member of Pollonera's (1887) *Ambigolimax*, as a synonym of *Limax tenellus* (= *Malacolimax tenellus* (Müller, 1774) (Stalažs and Dreijers 2016; Bonkowski and Kappes 2017). In 1926, Hesse designated *Ambigolimax valentianus* as the type species of *Ambigolimax* by monotypy.

In the USA, Gregg (1944) and others (Pilsbry 1948; Westervelt 1961; Hanna 1966) erroneously identified *A. valentianus* as *Limax marginata* Müller, 1774, *Lehmannia marginata* (Müller, 1774), or *Limax arborum*. Quick (1952) was the first to identify this species, from specimens collected in Glendale, California, as *Lehmannia poireri* Mabilie, 1883, a name later synonymized with *Limax* (*Lehmannia*) *valentianus* (Waldén 1961). In 2007, Beckmann (2007) resurrected and elevated Pollonera's *Ambigolimax* subgenus to generic status for *Ambigolimax valentianus*, which has since been adopted by many authors (e.g. Udaka et al. 2008; von Proschwitz 2009; Gargominy et al. 2011; Rowson et al. 2014a, 2014b; Steury and Pearce 2014; Parmakelis et al. 2017). Other authors have continued to use *Limax valentianus* (e.g. Matsuo et al. 2011), *Lehmannia valentiana* (e.g. Hayes et al. 2012; Burke and Leonard 2013; Stalažs and Dreijers 2016), and *Lehmannia poirieri* (e.g. Kabashima et al. 2014). Likewise, the publication year of this species' description by Férussac is reported by various authors as 1821 (e.g. Robinson 1999), 1822 (e.g. Gargominy et al. 2011), and 1823 (e.g. Barker 1999), an issue that will be addressed further in the Discussion.

Material and methods

Collection and specimen preparation

Slugs matching the general description of *A. nyctelius* and *A. valentianus* were collected by hand between 2016 and 2018 by J. Vendetti and SLIME project participants L. Carlton, A. Curran, C. Lee, and R. Matsumoto from sites in Los Angeles County including the campuses of the University of California Los Angeles (UCLA) and California State University Los Angeles (CSULA), residential neighbourhoods in the cities of Los Angeles, Alhambra, Burbank, Gardena, Pasadena, and West Covina, suburban parks in the cities of Beverly Hills (Coldwater Canyon Park), Los Angeles (Holmby Park), Monterey Park (Barnes Park), and Glendale (Casa Verdugo Adobe Park), two urban parks in Los

Angeles (the NHMLA Nature Garden and Griffith Park), and an industrial/commercial area at the border of the cities of Carson and Gardena. Slugs matching the general description of *A. nyctelius* and *A. valentianus* were also collected by hand from two urban parks in New York City: Central Park in Manhattan and Columbus Park in Brooklyn, by C. Lee in 2017 (Table 1). One slug, whose COI sequence is used in this analysis, was collected beneath a potted plant at a garden centre in Humboldt County, California by R. Mc Donnell on 10 October 2007. After the extraction and sequencing of COI from this specimen, both the specimen and DNA extract were accidentally discarded after being placed in storage.

All specimens collected in Los Angeles County or New York City were killed by drowning in still water for 12–24 h or carbonated water for 1–2 h, then transferred to and stored in 75–95% ethanol. All, except the Arcata specimen, have been deposited in the NHMLA Malacology Collection (with lot numbers preceded by LACM) (Table 1). Specimen observations posted to iNaturalist that coincide with collected specimens are denoted by ‘iNat’ followed by the unique observation number from that record’s URL, for example, ‘iNat 4936338’ for www.inaturalist.org/observations/4936338 (Table 1).

Radula and jaw preparation and reproductive anatomy dissection

To remove the jaw and radula, the buccal mass was dissected out of preserved slugs, after which the jaw was isolated and cleaned of tissue using fine forceps under a Wild M5A (Heerbrugg, Switzerland) or Nikon SMZ1000 (Tokyo, Japan) stereomicroscope. After the jaw was removed, each buccal mass was immersed in 2–3 ml of 5 M NaOH in a glass Petri dish (diameter: 50 mm) for 3–4 days to dissolve tissue around the radula, before final removal of any adhering tissue using fine forceps and/or a dog whisker under microscopy. Radulae were then washed in clean water. Cleaned jaws and radulae were mounted on a penny covered with conductive tape, then adhered to a scanning electron microscope (SEM) stub. Reproductive anatomy was examined in preserved slugs by opening the body cavity near the pneumostome and removing the genitalia. Identification of reproductive parts followed Mc Donnell et al. (2009) and Rowson et al. (2014b).

Scanning electron microscopy and photography

Prepared jaws and radulae were coated in 60% gold, 40% palladium (@ 0.014 kÅ) by an Emitech K550x sputter coater (Ashford, UK) and imaged using a Hitachi S-3000N SEM (Tokyo, Japan) at an accelerating voltage of 15 kV and working distance of 12.9–13.1 mm in the SEM laboratory at NHMLA. A Nikon d7200 digital camera was used to take images of slug jaws before sputter coating. Digital images were adjusted in Preview v. 8.0 in MacOS X for contrast and brightness. An Apple iPhone (Cupertino, CA, USA) was used to photograph slugs while alive and after dissection.

DNA extraction, sequencing, and submission to GenBank

Total genomic DNA was extracted from foot tissue clippings of preserved slugs using the Qiagen DNeasy® Blood and Tissue Kit (Valencia, CA, USA) following the manufacturer’s instructions. PCR amplification used universal invertebrate primers for mitochondrial DNA

Table 1. Specimen and COI barcode sequence information for species in the family Limacidae referenced herein. GenBank accession numbers in bold indicate COI sequences generated by this study.

NHMLA . . no.	Collector	Collection date or (submission date)	Genbank or BOLD acc. no.	iNat no.	Collection locality
<i>Ambigolimax nyctelius</i> (Bourguignat, 1861)					
181211	Lee	12-Mar-2016	MG747684		USA: California, L.A. Co., Los Angeles, Nature Gardens, NHMLA
179183	Vendetti	13-Mar-2016	MG747683		USA: California, L.A. Co., Los Angeles, CSULA campus
181212	Carlton	14-May-2016	MG747682		USA: California, L.A. Co., Pasadena, N. El Molino Ave and E. Penn St.
179154	Lee	19-Jun-2016	MG799133	3494974	USA: California, L.A. Co., Los Angeles, Griffith Park
180640	Lee	08-Jan-2017	MG799137	4923018	USA: California, L.A. Co., Monterey Park, Barnes Park
180544	Lee	11-Jan-2017	MG747671	4936339	USA: California, L.A. Co., Los Angeles, UCLA campus
180545	Lee	11-Jan-2017	MG747679	4936337	USA: California, L.A. Co., Los Angeles, UCLA campus
180542	Lee	11-Jan-2017	MG747672	4936338	USA: California, L.A. Co., Los Angeles, UCLA campus
180543	Lee	11-Jan-2017	MG747673	4936336	USA: California, L.A. Co., Los Angeles, UCLA campus
180541	Lee	11-Jan-2017	MG747675	4936344	USA: California, L.A. Co., Beverly Hills, Coldwater Canyon Park
180540	Lee	11-Jan-2017	MG747677	4936343	USA: California, L.A. Co., Beverly Hills, Coldwater Canyon Park
180539	Lee	11-Jan-2017	MG747680	4936342	USA: California, L.A. Co., Beverly Hills, Coldwater Canyon Park
180635	Lee	13-Jan-2017	MG799136	4941396	USA: California, L.A. Co., Los Angeles, Cashmere St. and Montana Ave.
180636	Lee	08-Feb-2017	MG799138	5077952	USA: California, L.A. Co., Los Angeles, near UCLA campus
180634	Lee	11-Feb-2017	MG799135	5094179	USA: California, L.A. Co., Los Angeles, Mt. Washington neighborhood
179646	Matsumoto	14-Apr-2017	MG799140	5713872	USA: California, L.A. Co., Gardena, Bonsallo Ave. and S. Hoover St.
180631	Vendetti	08-Oct-2017	MG799134		USA: California, L.A. Co., Glendale, Casa Verdugo Adobe
180633	Lee	08-Jan-2018	MG799139	4923017	USA: California, L.A. Co., Monterey Park, Barnes Park
Lost	Mc Donnell	10-Oct-2007	MG856342		USA: California, Humboldt Co., Arcata, at a garden center
		12-Oct-2015	MOBIL1020-15.COI-5P		USA: California, San Diego Co., San Diego, 7555 Draper Ave.
		23-Nov-2013	KF894253		United Kingdom: Lanarkshire
		23-Nov-2013	KF894254		United Kingdom: Lanarkshire
		23-Nov-2013	KF894251		United Kingdom: Lanarkshire
		23-Nov-2013	KF894302		United Kingdom: South Devon
		23-Nov-2013	KF894303		United Kingdom: South Devon
		23-Nov-2013	KF894237		United Kingdom: South Devon
		23-Nov-2013	KF894238		United Kingdom: South Devon
<i>Ambigolimax valentianus</i> (Férussac, 1821)					
180632	Lee	13-Mar-2016	MG799143	5088308	USA: California, L.A. Co., Los Angeles, S. Bentley Ave. & Cashmere St.
179153	Curran	1-May-2016	MG799141	4467519	USA: California, L.A. Co., Burbank, at a garden center

(Continued)

Table 1. (Continued).

NHMLA no.	Collector	Collection date or (submission date)	Genbank or BOLD acc. no.	iNat no.	Collection locality
180546	Lee	16-Jun-2016	MG747681	3470667	USA: California, L.A. Co., West Covina, near Vista Rambla
179176	Lee	21-Jun-2016	MG799142	3504853	USA: California, L.A. Co., Alhambra, near Helman and S. Marengo Ave
180537	Lee	11-Jan-2017	MG747674	4936340	USA: California, L.A. Co., Los Angeles, Holmby Park
180536	Lee	11-Jan-2017	MG747676	4936341	USA: California, L.A. Co., Los Angeles, Holmby Park
180538	Lee	11-Jan-2017	MG747678	4936346	USA: California, L.A. Co., Los Angeles, Holmby Park
180641	Lee	11-Feb-2017	MG799144	5094181	USA: California, L.A. Co., Los Angeles, Mt. Washington neighborhood
180630	Vendetti	27-Sep-2017	MG799145		USA: California, L.A. Co., Gardena, near 17232 S Main St.
181202	Lee	28-Oct-2017	MG747685	8660679	USA: New York, N.Y. Co., New York, Central Park
180735	Lee	29-Oct-2017	MG747686	8671459	USA: New York, Kings Co., New York, Brooklyn, Columbus Park
		27-Jul-2012	JX435832		France: Aude, Alet-les-Bains
		2004	JX117876		Argentina
		14-Apr-2006	AM259710		United Kingdom: West Yorkshire, Leeds, Headingley
		14-Apr-2006	AM259711		United Kingdom: West Yorkshire, Leeds, Headingley
		23-Nov-2013	KF894276		Ireland: Mid Cork
		23-Nov-2013	KF894281		Ireland: West Galway
		23-Nov-2013	KF894290		United Kingdom: Antrim
<i>Bielzia coerulans</i> (Bielz, 1851)					
		27-Jul-2012	JX435829		Romania: Bucegi Mountains
		27-Jul-2012	JX435825		Romania: Fagaras Mountains
<i>Lehmannia marginata</i> (Müller, 1774)					
		05-Jan-2009	FJ606455		Sweden: Dalsland
		23-Nov-2013	KF894372		United Kingdom: Worcestershire
		23-Nov-2013	KF894368		United Kingdom: Glamorganshire
		23-Nov-2013	KF894377		United Kingdom: Midwest Yorkshire
<i>Limacus flavus</i> (Linnaeus, 1758)					
		23-Nov-2008	FJ481181		Not provided
		23-Nov-2013	KF894331		United Kingdom: Middlesex
<i>Limax cinereoniger</i> Wolf, 1803					
		2006	FJ606464		France: Bonneville, Rhone-Aples
		2006	FJ606465		Switzerland: Berne, Krauchthal
<i>Limax maximus</i> Linnaeus, 1758					
		27-May-2010	JN248294		Germany: Baruth
		14-July-2009	KM612139		Canada: Newfoundland
<i>Malacolimax tenellus</i> (Müller, 1774)					
		27-Jul-2012	JX435836		Poland: Silesian Lowlands
		23-Nov-2013	KF894369		United Kingdom: Worcestershire
		23-Nov-2013	KF894297		United Kingdom: West Kent
		23-Nov-2013	KF894379		United Kingdom: Northeast Yorkshire
		23-Nov-2013	KF894349		United Kingdom: Moray

gene cytochrome *c* oxidase subunit I (COI): LCO1490 and HCO2198 (Folmer et al. 1994). PCR used 12.5 µl of GoTaq® Green Master Mix (Promega; Madison, WI, USA) in 25 µl total volume reactions with 0.5–2.0 µl of isolated DNA, 0.5–1 µl of primers, 7–10 µl water, and 0.5–1 µl bovine serum albumin (0.1 µg µl⁻¹). Reaction cycles were as follows: 94°C for 2 min (denaturation), 40 amplification cycles at 40°C for 45 s (annealing), 72°C for 60 s (extension), with a final extension step of 72°C for 7 min. The presence of PCR products was confirmed by gel electrophoresis using 1% agarose gels containing EtBr. PCR products were purified and sequenced by Retrogen, Inc. (San Diego, CA, USA) in both directions using PCR primers. Resulting DNA fragments (as chromatograms) were inspected for quality, then aligned and trimmed of primers in Geneious® version 8.1.6 (Kearse et al. 2012). Sequences were submitted to GenBank with collection details (Table 1).

Sequence comparisons from GenBank and BOLD public databases

For comparison to sequences generated by the authors, GenBank and the Barcode of Life (BOLD) databases were searched for taxa from all genera within the Limacidae Lamarck, 1801 on 16 February 2018. The following partial COI sequences (Table 1) were downloaded from GenBank: seven of *A. nyctelius* (identified as *Lehmannia nyctelia*), acc. nos. KF894237, KF894238, KF894251, KF894253, KF894254, KF894302, KF894303, seven of *A. valentianus* (identified as *Ambigolimax valentianus*), acc. nos. AM259710, AM259711, KF894276, KF894290, KF894281, JX435832, JX117876, two of *Bielzia coerulans* (M. Bielz, 1851), acc. nos. JX435825, JX435829, two of *Limacus flavus* (Linnaeus, 1758), acc. nos. FJ481181, KF894331), four of *Lehmannia marginata* (Müller, 1774), acc. nos. KF894372, KF894368, FJ606455, KF894377, two of *Limax cinereoniger* Wolf, 1803, acc. nos. FJ606464, FJ606465, two of *Limax maximus* Linnaeus, 1758, acc. nos. JN248294, KM612139, five of *Malacolimax tenellus* (Müller, 1774), acc. nos. KF894369, KF894379, KF894297, KF894349, JX435836, and one from BOLD (identified as *Lehmannia nyctelia*), sample ID: BOLD-05VTCQ621, sequence ID: MOBIL1020-15.COI-5P (Table 1). No sequences of COI, or any other molecular marker, were available from either database for specimens within the following limacid genera: *Gigantomilax* O. Boettger, 1883, *Svanetia* P. Hesse, 1926, *Caspilimax* P. Hesse, 1926, *Turcomilax* Simroth, 1902, *Eumilax* O. Boettger, 1881, and *Metalimax* Simroth, 1896.

Bielzia coerulans is the only species in the genus *Bielzia* Clessin, 1887; it is native to the Carpathian Mountains of Central and Eastern Europe (Welter-Schultes 2012) and its phylogenetic placement within the Limacidae is presented and discussed in Nitz (2013). The limacid genus *Limax* Linnaeus, 1758 is species rich and has been extensively sampled and phylogenetically treated using COI by other authors (e.g. Nitz et al. 2009; Rowson et al. 2014a). Therefore, only two *Limax* species (*L. cinereoniger* and *L. maximus*) are included in this study.

Notably, the *A. nyctelius* sequence in BOLD (sequence ID: MOBIL1020-15.COI-5P) was generated by the Centre for Biodiversity Genomics at the University of Guelph, Ontario, Canada from a specimen collected in the city of San Diego, San Diego County, California on 12 October 2015. As BOLD indicates that this slug was collected in 2015, its discovery predates the detection and identification of *A. nyctelius* in Los Angeles County by approximately five months. After *A. nyctelius* was identified in Los Angeles County in 2016, the NCBI BLAST tool (Boratyn et al. 2013) was used to compare unpublished COI

sequences from *Ambigolimax* specimens collected by R. Mc Donnell from throughout California in 2007, to those in GenBank. One of those specimens was the 2007 Arcata, California slug, whose haplotype was identified as *A. nyctelius* (GenBank acc. no. MG856342) and is published here for the first time.

Sequence alignment, COI haplotype analysis, and phylogenetic inference

Thirty partial COI sequences generated by the authors and 32 from GenBank and BOLD databases (Table 1) were aligned in Geneious v. 8.1.6 using MUSCLE (Edgar 2004) and trimmed of primers, resulting in a total sequence length of 655 bases. The model of COI sequence evolution was chosen using Bayesian information criterion (BIC) with ModelFinder in IQ-TREE (Trifinopoulos et al. 2016; Kalyaanamoorthy et al. 2017) and jModelTest2 (Darriba et al. 2012) on XSEDE within CIPRES Science Gateway 3.3 (Miller et al. 2010).

FaBox DNACollapser (Villesen 2007) was used to group partial *A. nyctelius* and *A. valentianus* COI sequences into unique and/or shared haplotypes. Sequences were trimmed to 617 base pairs to minimize missing data and a final alignment was made using Consensus Alignment or MUSCLE in Geneious. Mean intra and interspecific COI haplotype genetic distances for the eight species included in this study were calculated in MEGA7 (Kumar et al. 2016) using the K2P model (Kimura 1980), default settings, and all codon positions.

The figured COI phylogram was generated using maximum likelihood by SATé (Simultaneous Alignment and Tree Estimation) 2.2.7 for Mac and the algorithms SATé-II (Liu et al. 2011), FastTree (Price et al. 2009), and a sequence evolution model chosen by SATé. Support values for the SATé phylogram were added at nodes also resolved by analyses of the same dataset using RAXML v. 8.2.10 (maximum likelihood) (Stamatakis 2014) and MrBayes v. 3.2.6 (Bayesian inference) Markov chain Monte Carlo (MCMC) (Ronquist et al. 2012) within CIPRES 3.3. RAXML analysis used the default settings of RAXML-HPC BlackBox for a 50% majority consensus tree using the HKY+ CAT sequence evolution model (Hodkinson and Parnell 2006). MrBayes was parameterized to produce a 50% majority consensus tree as the result of two runs of four Markov chains each of 1 million generations, using the HKY model of sequence evolution (HKY, $nst = 2$, rates = gamma, ngammacat = 6) (Hasegawa et al. 1985) and sampling every 1000 trees, with a burn-in fraction of 25%. Informative nodes are labelled with RAXML bootstrap values above and MCMC posterior probabilities below.

Results

External and internal morphology in *A. nyctelius* and *A. valentianus*

The mantle banding pattern of *A. nyctelius* and *A. valentianus* has been used by some authors to distinguish between them (Borredà and Martínez-Ortí 2017), but other authors report that mantle banding is variable and therefore insufficient for species identification (e.g. Welter-Schultes 2012). External features of specimens of *A. nyctelius* ($n = 18$) and *A. valentianus* ($n = 11$) collected for this analysis are summarized as follows: body slightly to moderately translucent; ground colour light brown/cream to amber brown; melanistic and pale colour forms not uncommon; mantle with fine fingerprint-like wrinkles; mantle patterning often with two lateral and one central band highly

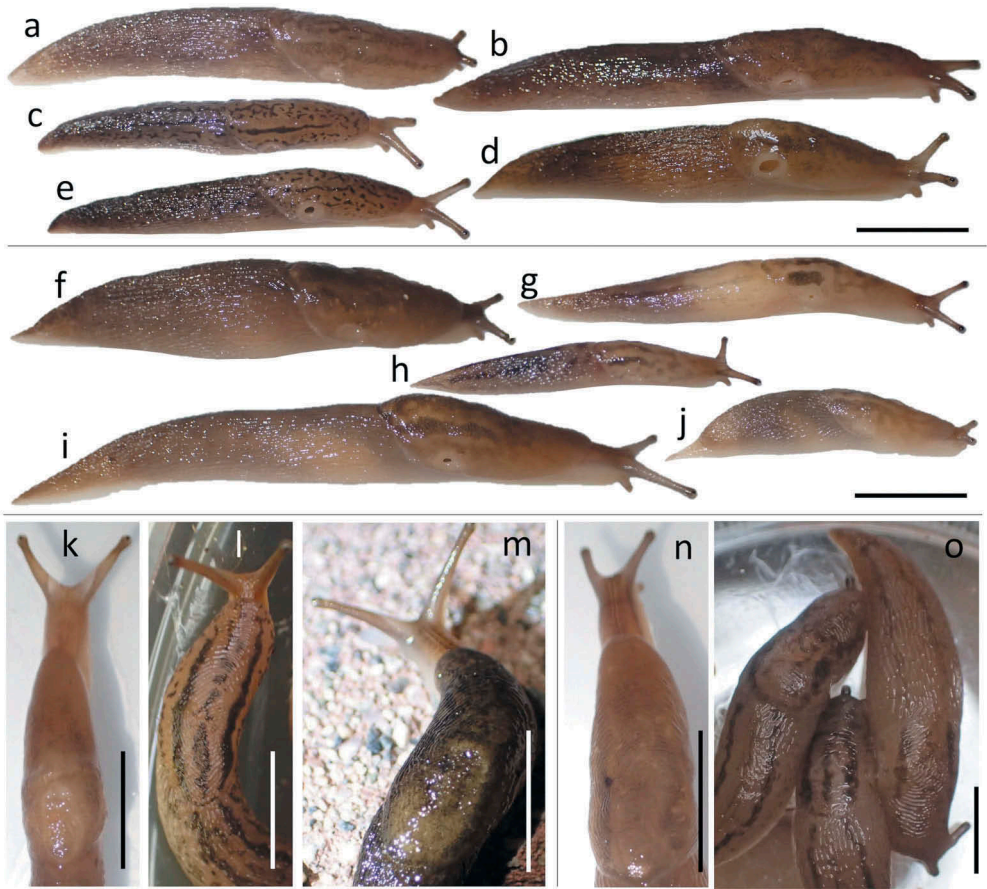


Figure 1. External phenotype of *A. nyctelius* (a–e, k–m) and *A. valentianus* (f–j, n–o) from sites within Los Angeles County, California. Scale bars = 1 cm. (a) iNat 4936343, LACM 180540; (b) iNat 4936344, LACM 180541; (c) iNat 4936336, LACM 180543; (d) iNat 4923019; (e) iNat 4936342, LACM 180539; (f) iNat 4936340, LACM 180537; (g) iNat 5088308, LACM 180632; (h) iNat 4941401; (i) iNat 4936341, LACM 180536; (j) iNat 4936346, LACM 180538; (k) iNat 4936338, LACM 180542; (l) iNat 3494974, LACM 179154; (m) iNat 5094179, LACM 180634; (n) iNat 4936340, LACM 180537; (o) iNat 3504853, LACM 179176. Photos by Cedric Lee.

variable from nearly absent to darkly pigmented and prominent or blotched; internal shell visible through mantle in some individuals; pneumostome encircled by a cream to tan coloured ring; tail often with ‘tram-lines’ running down its length with pigmentation absent, faint, blotched, or prominent; body length 2.5–5.5 cm (Figure 1(a–o)).

Jaws and radulae of *A. nyctelius* and *A. valentianus* specimens collected in Los Angeles County were not analysed statistically, but both appear to vary somewhat within species. Their characteristics are summarized as follows: jaw (Figure 2(a–h)) oxygnathic, variable in colour from tan to dark brown with a shallow median projection and variability in degree of jaw curvature and prominence of median projection, with concentric rings of variable thickness especially toward cutting edge, width 2.2–3 mm; radula (Figures 3 and 4(a–f)) with central tooth nearly symmetrical to moderately asymmetrical, tricuspid and lanceolate, mesocone elongate, pointed endocones and ectocones nearly half the length of total tooth, lateral teeth

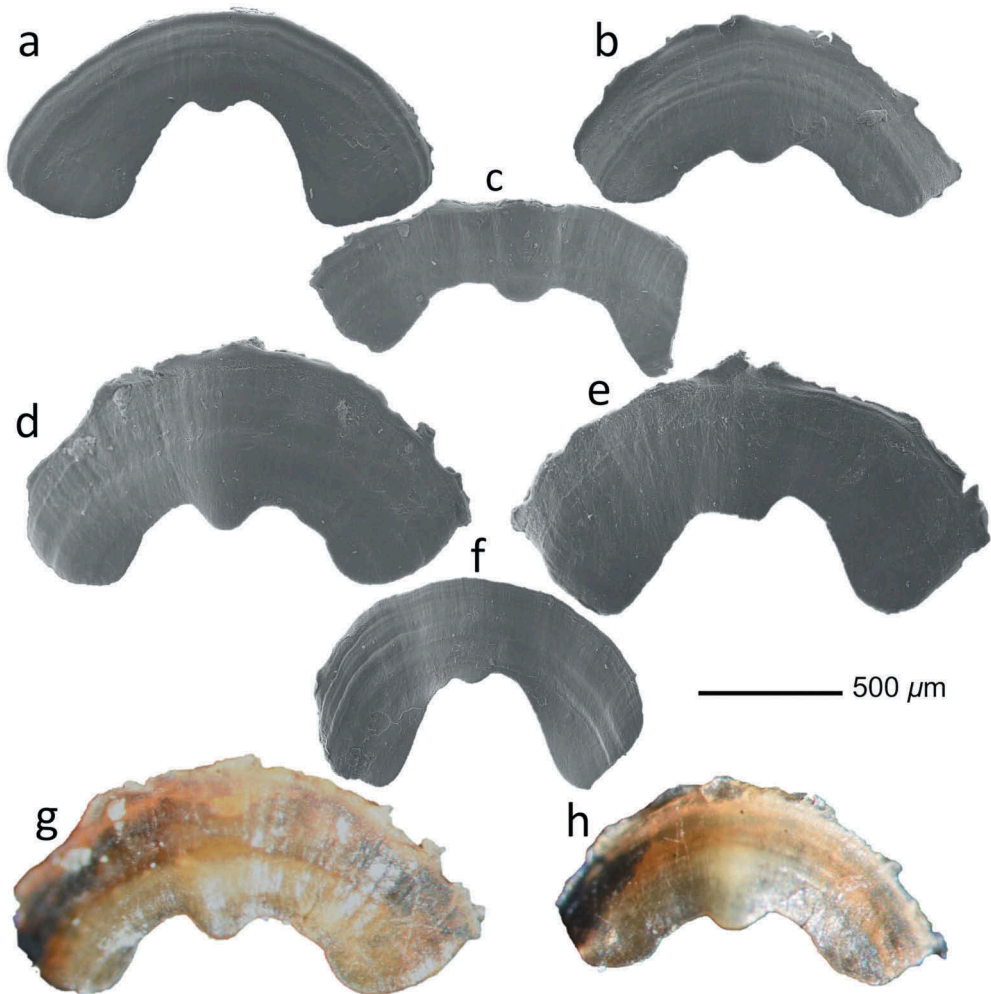


Figure 2. Jaws of *A. nyctelius* (a–c, h) and *A. valentianus* (d–g) from sites within Los Angeles County, California imaged using SEM (a–f) and light microscopy (g, h). (a) iNat 4936336, LACM 180543; (b, h) iNat 4936344, LACM 180541; (c) iNat 4936343, LACM 180540; (d, g) iNat 4936341, LACM 180536; (e) iNat 4936340, LACM 180537; (f) iNat 4936346, LACM 180538. Images by Emily Burnett.

tricuspid and lanceolate, slightly longer than central tooth, with pointed ectocones shorter than endocones, marginal teeth highly variable, broad with up to 6 tiny comb-like cusps most distally, narrow and sickle-shaped unicuspid and/or bicuspid more proximally; radula formulae and central tooth dimensions in *A. nyctelius* (LACM 180543), 125 transverse rows \times 1C + 15L + 37M, radula length 2.7 mm, central tooth length 28 μ m, central tooth width 13 μ m; (LACM 180540) central tooth length 30 μ m, central tooth width 15 μ m; in *A. valentianus* (LACM 180538), 115 transverse rows \times 1C + 15L + 39M, radula length 2.9 mm, central tooth length 29 μ m, central tooth width 10 μ m; (LACM 180536) central tooth length 37 μ m, central tooth width 12 μ m.

Phallus morphology was found to be diagnostic to species, as proposed by other authors (Quick 1960; Mc Donnell et al. 2009), and was consistent with species identities

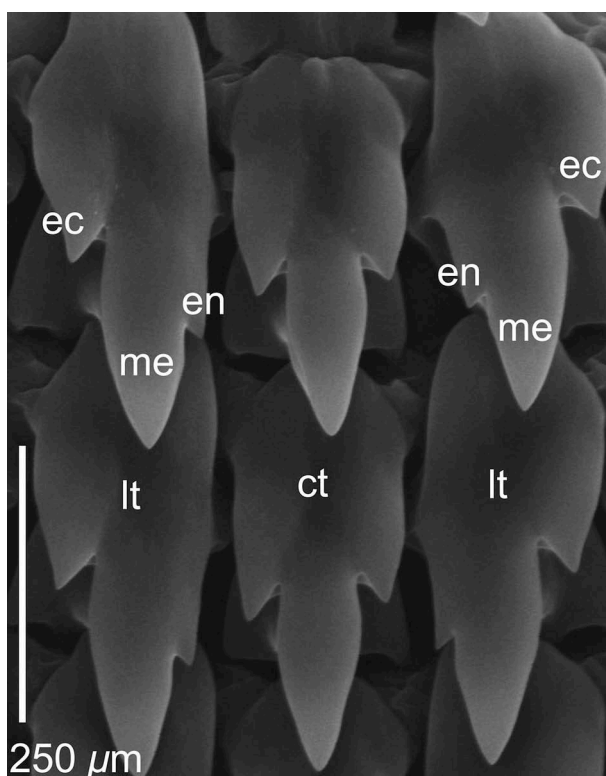


Figure 3. Radula of *A. valentianus* from Los Angeles County, California, LACM 2015-6.3. ct, central tooth; lt, lateral teeth; ec, ectocone, the tooth's outer cusp; en, endocone, the tooth's inner cusp; me, mesocone, the tooth's middle cusp. After Taylor et al. 1907 and Rumi et al. 2017. Image by Emily Burnett.

confirmed using COI barcoding sequences analysed independently and using the NCBI BLAST tool (Boratyn et al. 2013). In 17 examined specimens of mature *A. nyctelius*, the phallus had no appendix (Figure 5(a)); in 10 examined specimens of mature *A. valentianus*, the phallus had a bulbous and blunt-tipped appendix (Figure 5(b)).

Haplotype analysis and phylogenetic systematics of *A. nyctelius*, *A. valentianus*, and selected limacids

As of spring 2018, the submission of COI haplotypes for *A. nyctelius* and *A. valentianus* into GenBank from this study increases their numbers from 8 to 26 or 225% for *A. nyctelius*, and from 29 to 40 or 38% for *A. valentianus*. For the COI sequence alignment of *A. nyctelius*, *A. valentianus* and selected limacids, the chosen model of sequence evolution was HKY+ F+ G4 (with BIC) by IQ-TREE, HKY+ G by jModelTest2, and GTR+ G20 by SATé. Within 24 *A. nyctelius* COI sequences (trimmed to 618 bp), there were nine haplotypes and a mean intraspecific K2P genetic distance of 1.4%; for 15 *A. valentianus* COI sequences, there were eight haplotypes and a mean intraspecific K2P genetic distance of 0.4%. The two specimens collected from New York City parks were identified as *A. valentianus* by examination of phallus morphology and COI haplotype, and share similar haplotypes to specimens collected in Los Angeles. The number of differences between the two New York City-collected *A. valentianus* slugs was 6 out of 619 COI

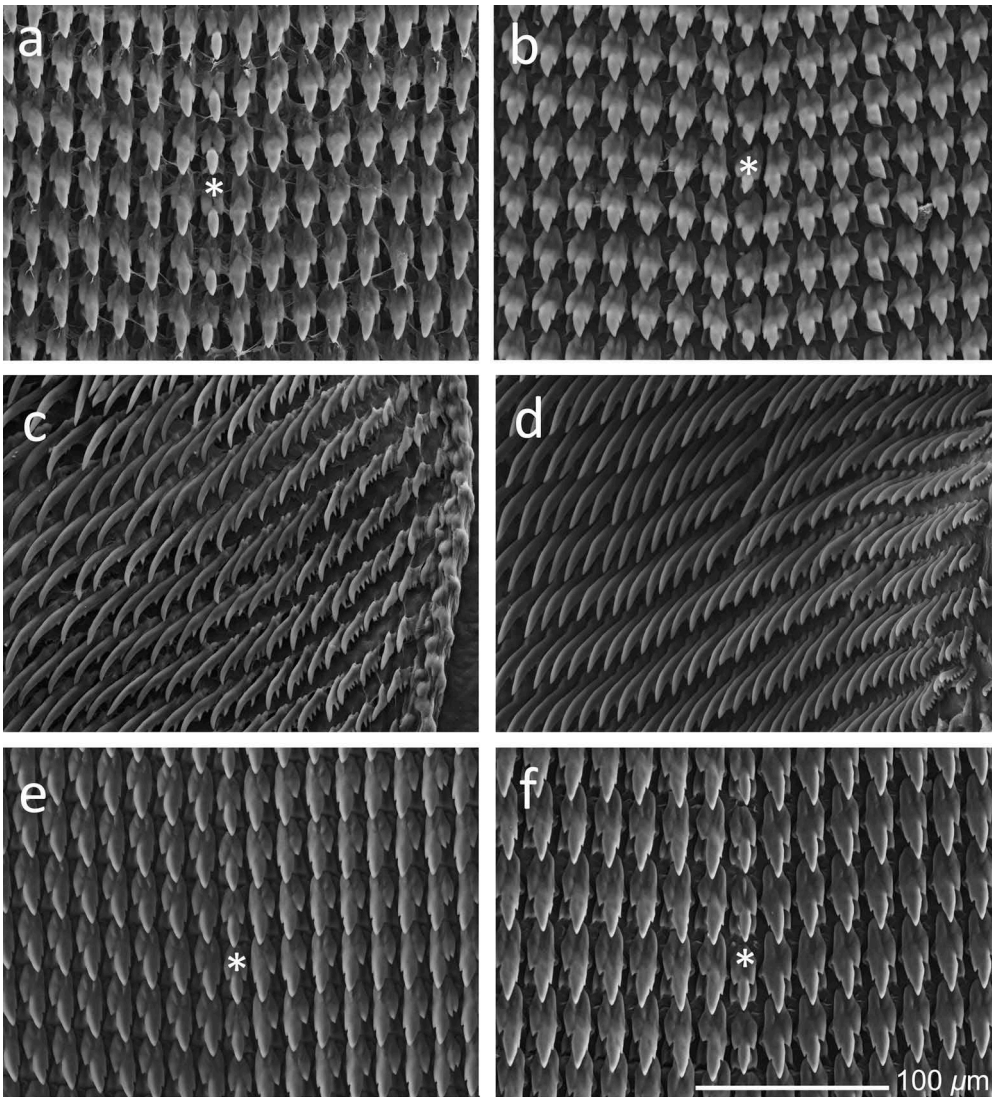


Figure 4. Radulae of *A. nyctelius* (a–c) and *A. valentianus* (d–f) from sites within Los Angeles County, California. (a) central and lateral teeth, iNat 4936343, LACM 180540; (b) central and lateral and (c) marginal teeth, iNat 4936336, LACM 180543; (d) marginal and (e) central and lateral teeth, iNat 4936346, LACM 180538; (f) central and lateral teeth, iNat 4936341, LACM 180536. An asterisk (*) indicates the vertical row of central teeth. Images by Emily Burnett.

base pairs. The range in number of nucleotide differences within COI for *A. valentianus* (trimmed to 617 bp) was 0–6; in *A. nyctelius* it was 0–30. Mean interspecific K2P COI genetic distance between *A. valentianus* and *A. nyctelius* for the haplotypes analysed is 8.6% (Table 2). There were some differences between phylogeny topologies produced by SATé, RAxML, and MrBayes methods (not shown), but the SATé-produced phylogram shown (Figure 6) represents the result of all analyses, in that *A. nyctelius*, *A. valentianus* and the other six species were resolved as monophyletic. All analyses also resolved *A. nyctelius* and *A. valentianus* as sister taxa (Figure 6), although the support values for *A. nyctelius* as monophyletic (bootstrap 89, posterior

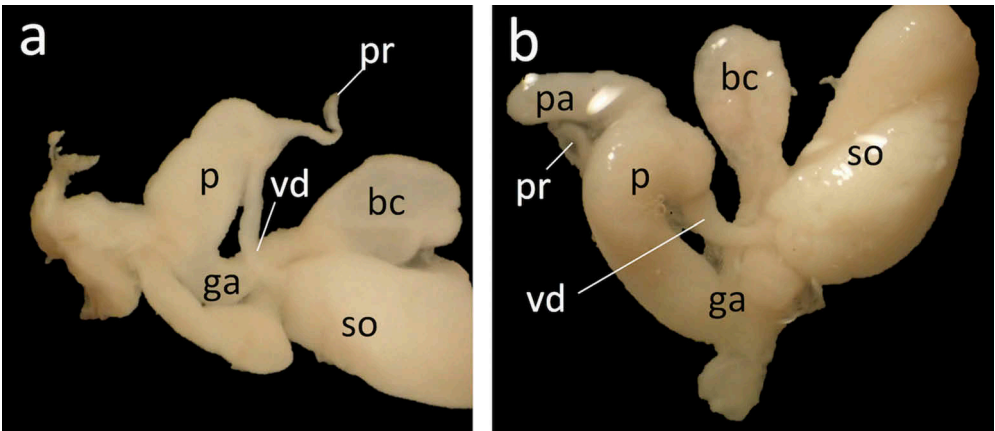


Figure 5. Male genitalia of *A. nyctelius* (a), iNat 4936338, LACM 180542, and *A. valentianus* (b), iNat 4936340, LACM 180537 from Los Angeles County, California. bc, bursa copulatrix; ga, genital atrium; p, phallus; pa, phallus appendix; pr, phallus retractor muscle; so, spermoviduct; vd, vas deferens. Photos by Cedric Lee.

prob. 0.93) are lower than for *A. valentianus* (bootstrap 100, posterior prob. 1). The lower nodal support for *A. nyctelius* as monophyletic are likely because of sequences KF894238 and KF894237 (in Rowson et al. 2014a; ‘DC2nyct and DY3 nyct’), which represent an identical haplotype with 17 single nucleotide polymorphisms not present in the other 25 *A. nyctelius* sequences analysed.

The COI sequence from one specimen collected in Arcata, California in 2007 (GenBank acc. no. MG856342) nests within one of three clades of *A. nyctelius*, and haplotypes from two New York City-collected *A. valentianus* nested within a polytomy of *A. valentianus* specimens collected within Los Angeles County. All analyses weakly resolved a sister relationship between *L. marginata* and *B. coeruleans* (Figure 6). Where support values are absent indicates a node that was unresolved in all analyses or had a very low support value (<40 bootstrap, <.50 posterior prob.).

Discussion

Under-reported biodiversity and potential for citizen science

Stylommatophoran biodiversity in both natural and human-altered habitats is commonly under-reported (Reise et al. 2006; Forsyth 2014; Araiza-Gómez et al. 2017). For example, in the last 20 years, detection surveys for introduced terrestrial slugs have produced new

Table 2. Estimated mean inter and intraspecific (K2P) genetic distance of COI haplotypes in eight limacid slug species. Intraspecific genetic distances are indicated in bold. Standard error estimates range from 0.013–0.025.

	<i>A. val</i>	<i>A. nyc</i>	<i>M. ten</i>	<i>L. cin</i>	<i>L. max</i>	<i>Le. mar</i>	<i>B. coe</i>	<i>L. fla</i>
<i>A. valentianus</i> (n = 15)	0.005							
<i>A. nyctelius</i> (n = 24)	0.086	0.021						
<i>M. tenellus</i> (n = 5)	0.162	0.156	0.006					
<i>L. cinereoniger</i> (n = 2)	0.200	0.200	0.159	0.000				
<i>L. maximus</i> (n = 2)	0.182	0.186	0.173	0.148	0.000			
<i>Le. marginata</i> (n = 4)	0.171	0.164	0.181	0.202	0.192	0.005		
<i>B. coeruleans</i> (n = 2)	0.156	0.159	0.156	0.230	0.221	0.168	0.027	
<i>L. flavus</i> (n = 2)	0.158	0.164	0.180	0.191	0.202	0.192	0.180	0.000

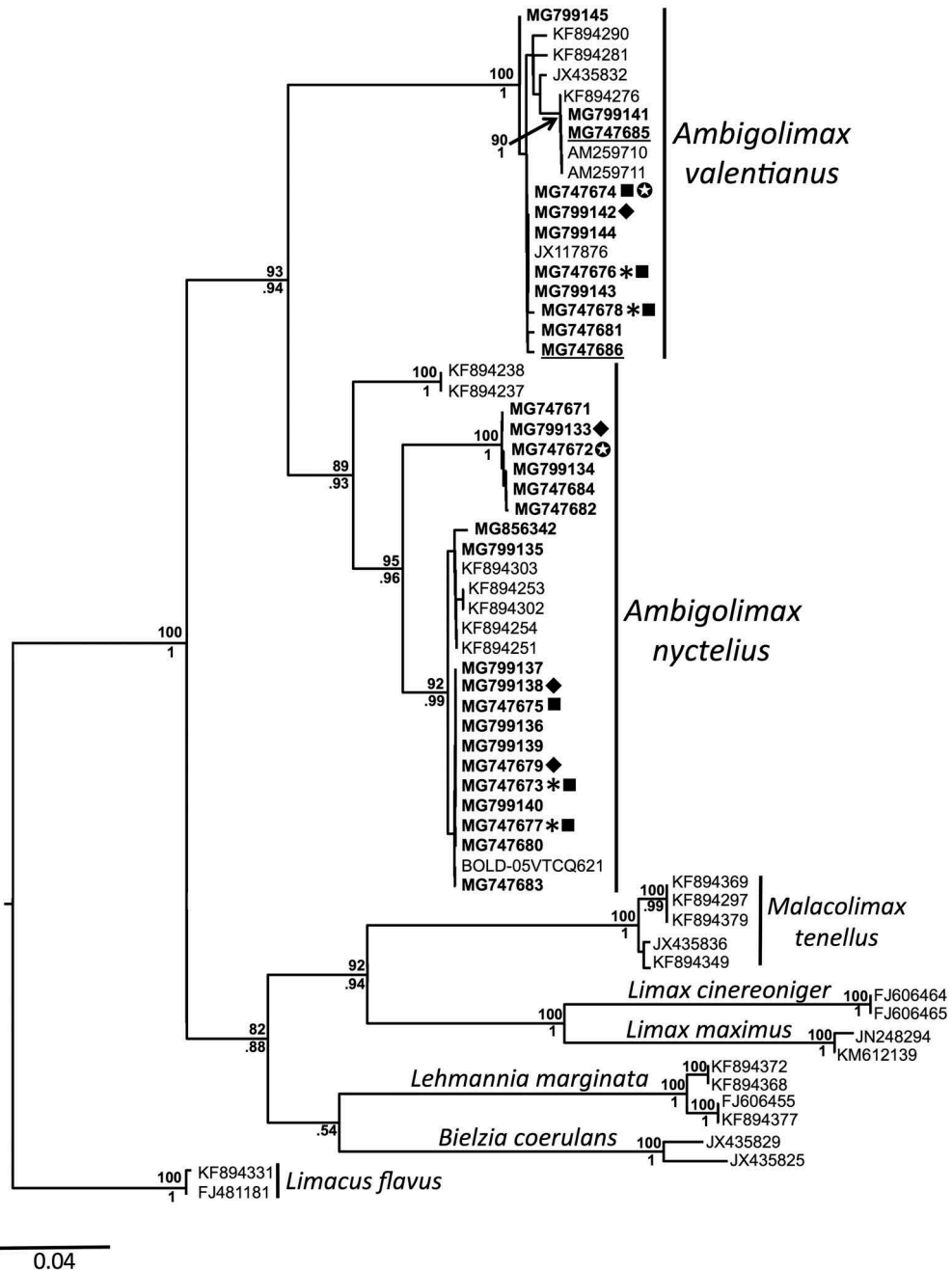


Figure 6. Phylogram based on a SATé FastTree analysis of aligned, partial COI mtDNA sequences from selected limacid gastropod species. Specimens are represented by their GenBank accession number with the following indications: bold = COI sequence generated by this study, boxed = first sequence of *A. nyctelius* from a specimen collected in California, underlined = collected in New York City, asterisk = radular morphology shown in Figure 4, diamond = external phenotype shown in Figure 1, square = jaw morphology shown in Figure 2, a star within a circle = distal male genital morphology shown in Figure 5. RAxML bootstrap support values are shown above and next to nodes, and MrBayes posterior probabilities are below. Where support values are absent indicates a node that was not resolved by all analyses or a node with very low support values.

species records worldwide (Europe: Roques et al. 2009; Stojnić et al. 2011; Rowson et al. 2014a; Asia: Wiktor et al. 2000; Oceania: Brodie and Barker 2011; Africa: Rowson et al. 2016; South America: Gregoric et al. 2013; Hausdorf 2002; Gomes et al. 2011; Araya 2015; North America: Reise et al. 2006; Moss and Hermanutz 2010; Mc Donnell et al. 2014; Araiza-Gómez et al. 2017). In western North America, the native slug *Anadenulus cockerelli* (Hemphill, 1890) was recently rediscovered in San Diego County, California after having gone unreported for more than 50 years (Richart et al. 2018), and a new endemic arionid slug, *Hemphillia skadeii* Lucid et al., 2018, was recently described from the Pacific Northwest where known species diversity lags behind expected species diversity (Frest et al. 2001; Lucid et al. 2018). Globally, the under-reporting of introduced slug species may have damaging consequences for native flora, agriculture, and ranching, as many non-native taxa are destructive to crops and some are vectors for plant or animal disease (Gismervik et al. 2015; Sharadraj and Chandra Mohanan 2015). Thus, regular surveys of land snails and slugs and accurate knowledge of regional stylommatophoran biodiversity, native and introduced, could inform conservation policy and land management (Bros et al. 2016), improve protocols for import and export quarantine, update strategies for species eradication (Cowie et al. 2009), and reduce detection time for invasives (Maistrello et al. 2016).

As demonstrated with other taxa, including lizards (Pauly et al. 2014) and birds (Callaghan et al. 2017), citizen science-based approaches can greatly improve faunal inventories and increase species records of urban-living taxa. For example, using citizen science observations, the limacid slug *L. maximus* was documented in and around metropolitan areas in the UK and Japan (Bates et al. 2015; Morii and Nakano 2017). In southern California, SLIME has amassed nearly 1200 observations of *Ambigolimax* slugs since 2015, approximately 85% of which were documented within the city of Los Angeles. Notably, many of these observations were made on private property that would otherwise be inaccessible for surveying, making the public's participation crucial to faunal inventories. Also, citizen science initiatives like SLIME would be relatively easy to reproduce elsewhere, are an effective means of institutional outreach, and can benefit participants by improving their understanding of science and enriching their engagement with nature and their community (Bonney et al. 2016; McKinley et al. 2017).

Systematics of *A. nyctelius* and *A. valentianus* and selected limacids

Ambigolimax nyctelius and *A. valentianus* collected from Los Angeles County could not be reliably differentiated by mantle patterning or the morphology of the radula or jaw, a finding that supports the conclusion of authors including Mc Donnell et al. (2009) and Rowson, Turner, et al. (2014b). These taxa could be differentiated by distal genital morphology and COI barcode sequences (Nitz et al. 2010; Rowson et al. 2014a). Such sequence data are not without limitations (Davison et al. 2009; Sauer and Hausdorf 2012), but are extremely useful for specimen identification, especially of phenotypically similar taxa such as *A. nyctelius*, *A. valentianus* and *L. marginata*. Notably, COI barcode analysis would also allow for distinguishing between these and other taxa non-lethally (Morinha et al. 2014) or when distal male genitalia are under-developed, as in juvenile slugs.

Sequence analysis herein revealed distinct COI haplotypes for *A. nyctelius* and *A. valentianus* that were not shared between species. *Ambigolimax nyctelius* haplotypes were more divergent than those of *A. valentianus*, corroborating findings of Rowson

et al. (2014a) who examined specimens collected in Europe. An 8.6% mean K2P inter-specific COI divergence between the two *Ambigolimax* species is relatively low compared to other slug species pairs (Rowson et al. 2014a) and is the lowest between any two limacid taxa examined in this study (Table 2). However, Nitz et al. (2010) found a comparatively low K2P COI distance of 10.8% between *Limax* species.

Both maximum likelihood and Bayesian inference analyses of COI (Figure 6) show all eight species, represented by sequence data from at least two specimens, as monophyletic. Notably, in all analyses *A. nyctelius* and *A. valentianus* are resolved as sister taxa. However and importantly, taxon sampling is too poor to conclude that *A. nyctelius* and *A. valentianus* diverged most recently from a common ancestor. There are 15 additional described species in the genus *Lehmannia* (Welter-Schultes 2012) and three in *Malacolimax*, all of which lack sequence data in BOLD and GenBank databases. No study, including this one, has examined sequence data of COI or any other molecular marker for species in other limacid genera, including six in *Gigantomilax*, seven in *Turcomilax*, three in *Eumilax*, two in *Metalimax*, and one each in *Svanetia* and *Caspilimax* (MolluscaBase 2018). This lack of data makes sequence-based inference of phylogenetic relationships within and between most genera within the Limacidae (e.g. *Ambigolimax*, *Lehmannia*, *Malacolimax*, *Bielzia*), and any conclusions about trait evolution, presently untenable.

Finally, in a literature search of the species name ‘valentiana’ or ‘valentianus’ referring to a limacid slug, a sample of 40 publications from 1999 to 2018 included 33 unique first authors who used the genus name *Ambigolimax* nine times, *Lehmannia* 23 times, and *Limax* eight times. This generic lability reveals the need for a revision of what we refer to here as *Ambigolimax* spp. For example, our results and those reported elsewhere (Klee et al. 2005; Rowson et al. 2014a) indicate that the genus *Lehmannia* is not monophyletic when its name is applied to *A. nyctelius* and/or *A. valentianus*. Additionally, Pollonera (1887) proposed *Ambigolimax* for species that have a globular phallus appendix, as in *A. valentianus*, but not in *A. nyctelius*. It is, therefore, problematic that the genus name *Ambigolimax* is applied here and elsewhere to *A. nyctelius*, especially as *A. valentianus* is the type species of the genus. Consequently, and because of the poor taxon sampling within *Lehmannia*, *Malacolimax*, and most other limacid genera (excluding *Limax*), we recommend a revision of the genus *Ambigolimax* after a thorough examination of molecular and morphological data from a diversity of limacid species. A resulting taxonomy could name a new genus for *A. nyctelius*, reject *Ambigolimax* altogether in favour of a new or different genus, or re-describe *Ambigolimax* without reference to the morphology of the phallus appendix.

Introduction of *A. nyctelius* and *A. valentianus* where non-native

Because *A. nyctelius* could be easily confused for *A. valentianus*, we speculate that *A. nyctelius* is likely established elsewhere in North America (possibly in New York City, but this was not borne out by our 2017 survey), and could have been introduced and/or established in California prior to its discovery in 2007 in Humboldt County. Likewise, *A. nyctelius* could have been introduced into Los Angeles County one or multiple times prior to its discovery in 2016. Because both species are introduced, the different degrees of genetic variation in COI within and between *A. nyctelius* and *A. valentianus* could be the result of a genetic bottleneck, or admixing from different source populations. The founder effect, common in introduced species (Tsutsui et al. 2000; Puillandre et al. 2008), would likely result in lower intraspecific

genetic distance, i.e. a genetic bottleneck. In contrast, admixing in introduced species (Kolbe et al. 2004; Hahn and Rieseberg 2017; but see Havill et al. 2016; Trucchi et al. 2016) may lead to greater genetic variability by the interbreeding of populations from multiple sources. Genetic analysis of *Ambigolimax* spp. individuals from within their native habitat as well as from different regions where they are synanthropic (and could also be source populations) would help to clarify these dynamics.

Species publication date of *Ambigolimax valentianus*

Just as three different genus names have recently been applied to *A. valentianus*, so have three different years been acknowledged as the publication date of Férussac's original description (Férussac and d'Audebard 1821). In the literature search described above, the dates assigned to Férussac's *Limax valentianus* by various authors are: 1821 (four times), 1822 (15 times), 1823 (seven times), and unattributed (14 times). These discrepancies and those of at least one other species with Férussac's authorship, *Limax alpinus* as authored in either 1821 (Kennard and Woodward 1926) or 1822 (Nitz et al. 2009), stem largely from publishing date omissions or inconsistencies in the various issues, called livrasions, of the folio and quarto editions of the French volumes in which they were published. These works are, 'Tableaux systématiques des animaux mollusques classés en familles naturelles, dans lesquels on a établi la concordance de tous les systèmes; suivis d'un prodrome général pour tous les mollusques terrestres ou fluviatiles, vivants ou fossils' (hereafter 'the Prodrome') and its illustrated companion with accompanying text, the 'Histoire naturelle générale et particulière des Mollusques terrestres et fluviales' (hereafter 'the Histoire') (Kennard 1942a, 1942b, 1942c).

The discrepancy in year attribution is exemplified within the quarto edition of the Prodrome owned by the American malacologist William Healey Dall, (www.biodiversitylibrary.org/bibliography/10558#/summary). In it, the date of publication is handwritten as 1822 and crossed out and faintly re-written as 1821. The first description of *Limax valentianus* is found within Dall's copy of the Prodrome on page 21 of a supplement called, 'Tableau Systématique de la Famille Des Limaces, Limaces, servant de supplement provisoire A Notre Histoire Naturelle de ces Animaux' (hereafter 'the Tableau Limaces'), which is printed with 'Juin 1821' [June 1821], and, according to Kennard (1942b), is printed with January 1821 in the folio edition. Figured specimens do not accompany this species description but are cited in it as 'Hist., pl. VIII A, Figures 5 and 6', which refers to the Histoire. Of the 1821 dates printed in the Tableau Limaces, Kennard (1942b) writes as follows, 'These are probably the dates of printing [...] whereas Férussac says that Prodrome Limaces [= Tableau Limaces] was issued on livr. 16 (Exp. Planch. supp. Plate 4A) and the date is 16 July 1822'. What Kennard refers to here is 'l'explication des planches supplémentaires', or the explanation of the supplementary plates. However, Kennard's statement appears to be incorrect, as the 'Exp. Planch. supp. Plate 4A' he cites does not refer to livraison 16, but to plates for *Limax antiquorum* Férussac and *Limax alpinus* Férussac (Figure 7).

Furthermore, Deshayes, in his continuation of Férussac's work (in a livraison of the Histoire dated 1823), refers to the publication year of the Tableau Limaces as 1821 in at least two instances reproduced and translated as follows, '(1) Tableau systématique de la famille des Limaces (Juin 1821), faisant partie de l'ouvrage de Férussac, intitulé: Tableaux systématiques des animaux mollusques classes en familles naturelles, p. 1 à 27.' [(1) Systematic table of the slug family (June 1821), part of Férussac's work, entitled: Systematic tables of classified mollusc

PLANCHE 4 A.

***Limax antiquorum*, FÉRUSSAC.**

Fig. 4-4. Diverses phases de l'accouplement de cette espèce. Ces figures, empruntées à l'*Isis*, 1819, sont données à l'appui d'observations faites par M. Werlich, et rapportées à la p. 96, γ.

***Limax alpinus*, FÉRUSSAC.**

5. Animal de grandeur naturelle, vu de profil.

6, 7. Le même, vu en dessus.

Figure 7. Explanation of the supplementary plates, or 'Exp. Planch. supp. Plate 4A' (Férussac et al. 1820–1851).

animals in natural families, p. 1 to 27.], and, 'le supplement provisoire que nous avons publié au mois de juin 1821 a apporté de grands changements à la partie systématique de notre Histoire de la famille des Limaces' [the provisional supplement that we published in June 1821 brought great changes to the systematic part of our History of the Limacid family] (Férussac and Deshayes 1820–1851). Therefore, in light of the printed date of 1821 on the quarto edition of the Prodrôme, Kennard's mistake regarding Férussac's reference to the publication of Tableau Limaces in livraison 16, and Deshayes' references to 1821 as the publication year of the Tableau Limaces, we propose that the year of Férussac's authorship of *Limax valentianus* should be recognized as 1821.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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