

Five new records of introduced terrestrial gastropods in southern California discovered by citizen science

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Abstract: Terrestrial gastropod inventories can be improved, both in scope and thoroughness, by including species observations made by citizen scientists. Few citizen science projects, however, focus on terrestrial gastropods and perhaps none has mobilized members of the public to survey the malacofauna of a major North American metropolitan area. Here we report first occurrence records of five introduced terrestrial gastropod species in the metropolitan areas of Los Angeles, Orange, and Riverside counties in California, discovered by citizen science: *Arion hortensis* Férussac, 1819, *Cochlicella barbara* (Linnaeus, 1758), *Lauria cylindracea* (Da Costa, 1778), *Pupoides albilabris* (C.B. Adams, 1841), and *Xerotricha conspurcata* (Draparnaud, 1801). Four of these taxa are known elsewhere in California and one, *L. cylindracea*, is a first occurrence record for the U.S.A. All were contributed to SLIME, a citizen science project and malacofaunal inventory of southern California initiated by the Natural History Museum of Los Angeles County and hosted online by iNaturalist. Species identifications were made based on snail or slug morphology and collected specimens' COI barcoding sequences, which were compared to those in GenBank and BOLD databases. These discoveries demonstrate the efficacy of SLIME and the potential for molluscan-focused citizen science to detect and document land snail and slug taxa in a major metropolis.

Key words: Los Angeles, urban biodiversity, bioblitz, land snail, community science

Public participation in scientific research (Bonney *et al.* 2009, Theobald *et al.* 2015), known as citizen or community science (Bonney *et al.* 2014, Eitzel *et al.* 2017), allies the public with scientists in a collaborative pursuit of scientific data. Projects with a contributory design accelerate the collection of biodiversity data through voluntary participation of non-specialists or non-professionals who make photographs of the natural world available to researchers (Dickinson *et al.* 2012, Shirk *et al.* 2012, Bonney *et al.* 2014). These photographs, as species observations, are often taken with smartphones that tag each image with precise location coordinates. When these images are contributed to online biodiversity platforms like eBird, iSpotnature, or iNaturalist they can become valuable to biologists as geo-referenced data points (Pimm *et al.* 2015, Bik 2017, Clark 2017). When biodiversity-themed contributory citizen science projects involve a natural history museum (NHM) there is an additional opportunity to collect, preserve, and house voucher specimens for research, exhibit, and education.

Here we report five first occurrence records of introduced terrestrial gastropod species in southern California that were documented by citizen science: *Arion hortensis* Férussac, 1819, *Cochlicella barbara* (Linnaeus, 1758), *Lauria cylindracea* (Da Costa, 1778), *Pupoides albilabris* (C.B. Adams, 1841), and *Xerotricha conspurcata* (Draparnaud, 1801). The discovery of *L. cylindracea* is the first vouchered record of this species in the U.S.A., *X. conspurcata* and *A. hortensis* are first records for Los Angeles County, *C. barbara* is a first record for Los Angeles and Orange counties, and *P. albilabris* is a first record for Riverside County, California. All were found in anthropogenically-altered habitats by contributors to SLIME (Snails and slugs Living in Metropolitan Environments), a malacofaunal survey initiated by the Natural History Museum of Los Angeles County (NHMLA) and hosted online by iNaturalist (www.inaturalist.org/projects/slime). Live-collected specimens were sequenced for the mtDNA barcoding gene COI, and preserved and deposited in the NHMLA Malacology collection. Specimen identifications were made based on

body and/or shell morphology and specimens' COI sequences were compared to those in GenBank and BOLD databases. Notably, four of these taxa, *A. hortensis*, *C. barbara*, *L. cylindracea*, and *X. conspurcata*, are considered "traveling species" by Robinson (1999), a designation for terrestrial gastropods that have been transported widely and are established beyond their native ranges.

Citizen science and mollusks – present efforts and potential

The most popular regional and worldwide citizen science projects with a contributory design (Shirk *et al.* 2012) and taxonomic focus (with >20,000 observations) document birds, reptiles and amphibians, charismatic insects, and mushrooms (*e.g.* ebird.org, herpmapper.org, e-butterfly.org, and mushroomobserver.org). Overall, molluscan-themed projects have been diverse but relatively few in number (Table 1), even though many molluscan groups would be well-suited to citizen science. Moreover, because terrestrial and freshwater gastropod biodiversity is under-reported in many parts of the

world (Reise *et al.* 2006, Araiza-Gómez *et al.* 2017), and species are regularly introduced by the commodities trade (Capinha *et al.* 2015), malacofaunal inventories could be more accurate, thorough, and easily updated by including volunteer surveyors and/or crowd-sourced data (Cohn 2008, Aravind 2013, Adriaens *et al.* 2015, USEPA 2016, Aravind 2017). Indeed, on public land, bioblitzes, or short-term biodiversity inventories (Cohn 1996), have successfully directed citizen scientists' effort towards detecting terrestrial and freshwater gastropods that are rare or newly introduced (Forsyth 2015, McAlpine *et al.* 2016, Topley 2017), live in poorly-surveyed regions (Pearce 2009, Van Devender *et al.* 2012), or are of conservation concern or pest status (Michalak and Price 2010).

With the addition of residents and property owners in surveying efforts, biodiversity inventories could, importantly, include species records from sites that would be otherwise inaccessible to scientists (*e.g.* residential backyards) (Ballard *et al.* 2017, Spear *et al.* 2017). In urban and suburban settings,

Table 1. Citizen science initiatives with a molluscan theme or that include mollusks. Projects are listed by their habitat emphasis (aquatic, marine, and terrestrial), in chronological order by project start year, and with names in bold indicating re-occurring or on-going surveys. All are contributory in design except ReClam the Bay, which was co-created (Shirk *et al.* 2012).

Project, habitat focus, and (duration)	Region	Species of focus	Website and/or relevant references
<i>Aquatic mollusk-focused</i>			
Distribution survey of <i>Pomacea canaliculata</i> (2017+)	Asia: Japan, Osaka and adjacent prefectures	<i>Pomacea canaliculata</i> (Lamarck, 1822)	sites.google.com/site/sukumiringo
<i>Marine mollusk-focused</i>			
Ellenic Network of Aquatic Invasive Species: ELNAIS (2001+)	Europe: Coast of Greece and Greek islands	Any marine organism, including gastropod <i>Aplysia dactylomela</i> Rang, 1828	elnais.hcmr.gr, Zenetos <i>et al.</i> 2013, Zenetos <i>et al.</i> 2015, Poursanidis and Zenetos 2013
Cape Cod National Seashore's Estuarine Monitoring Program (2005)	North America: Massachusetts, Cape Cod National Seashore	Mostly gastropods and bivalves including <i>Mya arenaria</i> Linnaeus, 1758	Thelen and Thiet 2008
ReClam the Bay (2005+)	North America: Coast of New Jersey	Bivalves including <i>Mercenaria mercenaria</i> (Linnaeus, 1758)	Bonney <i>et al.</i> 2009a reclamationthebay.org
Reef Check California (2006+)	North America: California coast	>30 species, including gastropod <i>Megastraea undosa</i> (Wood, 1828)	www.reefcheck.org/california/ca-overview, Freiwald and Wisniewski 2015
<i>Xenostrobus securis</i> eradication in Avilés estuary (2015)	Europe: Avilés estuary in Asturias, Spain	<i>Xenostrobus securis</i> (Lamarck, 1819)	Miralles <i>et al.</i> 2016
GROC (Catalan Opisthobranch Research Group) (2016+)	Europe: Coast of Balearic Islands and Catalonia, Spain	Heterobranch gastropods including <i>Aplysia dactylomela</i> Rang, 1828	Moles <i>et al.</i> 2017, opisthobranchis.org/es/home
<i>Terrestrial mollusk-focused</i>			
Evolution MegaLab (2009+)	Europe: Various terrestrial habitats	<i>Cepaea nemoralis</i> (Linnaeus, 1758) and <i>Cepaea hortensis</i> (Müller, 1774)	evolutionmegalab.org, Silvertown <i>et al.</i> 2011, Cameron 2013
SLIME: Snails and slugs Living in Metropolitan Environments (2015+)	North America: southern California, especially urban areas	Terrestrial gastropods	www.inaturalist.org/projects/slime

these and other anthropogenically-altered localities (e.g. parks, vacant lots, school campuses) have great potential for synanthropic gastropod discovery as they are at the intersection of people who can make species observations and the habitat of native and/or introduced species (Haaland and van den Bosch 2015, Spear *et al.* 2017). Awareness of synanthropic terrestrial gastropod populations is also useful to researchers and pest management agencies, as scientific interest in the responses of species to urbanization has grown in recent years as well as concern about the introduction of pestiferous taxa (Beninde *et al.* 2015, Ives *et al.* 2016, McDonnell and MacGregor-Fors 2016, Alberti *et al.* 2017, Brown and Hartop 2017, Johnson and Munshi-South 2017, Kesner and Kumschick 2018).

SLIME at NHMLA

Snails and slugs Living in Metropolitan Environments (SLIME) began at NHMLA in August 2015 as a contribution-based citizen science inventory of land snails and slugs in southern California. Its geographic scope spans the counties of Imperial, Kern, Orange, Los Angeles, San Bernardino, San Diego, San Luis Obispo, Santa Barbara, Riverside, and Ventura. Species observations are contributed as photos through iNaturalist, and/or voucher specimens are accepted by the NHMLA Malacology collection. SLIME was modeled after another NHMLA-sponsored citizen science project called RASCals (Reptiles and Amphibians of southern California, Pauly *et al.* 2014, Pauly *et al.* 2015) that is also hosted on iNaturalist (www.inaturalist.org/projects/rascals). SLIME has been promoted to the public using social media (NHMLA on Facebook and Twitter), a blog (www.nhm.org/nature), yearly bioblitz events (e.g. SnailBlitz 2018), and multi-annual community meet-ups coordinated by NHMLA's Community Science Program. As a result of SLIME and the efforts to promote it, biologists at NHMLA have the infrastructure to detect recent terrestrial gastropod introductions in the region.

MATERIALS AND METHODS

Specimen collection and identification

The five terrestrial gastropod species discussed herein (Table 2) were collected actively by the following co-authors and citizen scientists, D.E., K.H., E.H., G.H., I.H., C.K., P.L., C.L., C.M., S.N., E.P., I.S., and J.E.V., and passively by the Lascano family whose backyard hosted a Malaise trap for the BioSCAN (Biodiversity Science: City and Nature) project, an NHMLA-sponsored insect collecting survey (Brown *et al.* 2014, Hartop *et al.* 2015) that inadvertently trapped *X. conspurcata* snails. All collected gastropod specimens were deposited in NHMLA's Malacology collection and catalogued with lot numbers preceded by LACM. Specimen or species

observation photo vouchers posted to iNaturalist are designated herein by "iNat" followed by the unique number of that observation's url (e.g. iNat 2602677 for www.inaturalist.org/observations/2602677).

Species identification of collected terrestrial gastropod specimens and iNaturalist photo vouchers were made or confirmed by co-authors J.E. Vendetti, C. Lee, and Malacology collections manager L. Groves, based on shell characters and/or external body morphology following the species accounts in Welter-Schultes (2012), Grimm *et al.* (2009), and Kerney and Cameron (1979). For slugs, a tentative identification of *Arion* sp. was replaced by *A. hortensis* after DNA was compared to arionid records in GenBank and BOLD databases and/or reproductive morphology was examined by co-author C. Lee and interpreted following Mc Donnell *et al.* (2009) and Rowson *et al.* (2014b).

Specimen microscopy and photography

Arion hortensis slugs intended for dissection were killed by drowning in water for 4–6 hours, then transferred to and stored in 75% ethanol. Dissections were performed using a Wild Heerbrugg M5A (Switzerland) or Nikon SMZ1000 (Japan) stereomicroscope. Scanning electron microscopy (SEM) was used to image the granular micro-protuberances on the apertural denticle of *L. cylindracea*. One shell (LACM 179780) was mounted on an SEM stub with conductive tape, coated with gold/palladium (60:40) at 0.014 kÅ by an Emitech K550x sputter coater (Kent, United Kingdom), and imaged with a Hitachi S-3000N SEM (Tokyo, Japan) at an accelerating voltage of 10 kV and working distance of 16.3 mm in the NHMLA Scanning Electron Microscopy Laboratory. A Keyence VHX-5000 digital microscope (Osaka, Japan) was used to photograph shells and shell details in *C. barbara*, *L. cylindracea*, *P. albilabris*, and *X. conspurcata*. Digital images and photomicrographs were adjusted in Preview v. 8.0 in MacOS X for contrast and brightness only.

DNA extraction, amplification, and sequencing

Foot tissue for DNA extraction was sampled from collected specimens of all five species after individuals were relaxed and killed by immersion in carbonated water for 1–2 hours then transferred to and stored in 95% ethanol. Total genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen Corp; Valencia, CA) following the manufacturer's instructions. Amplification by polymerase chain reaction (PCR) used universal invertebrate primers for the mitochondrial DNA gene cytochrome *c* oxidase subunit 1 (COI): LCO1490 and HCO2198 (Folmer *et al.* 1994). Amplification reactions used GoTaq® Green Master Mix (Promega; Madison, WI), a pre-mixed reagent solution for PCR, in 25µl total volume reactions with 1–2µl of isolated DNA, and proceeded as follows: a two-minute initial denaturation

Table 2. Taxon, NHMLA (LACM) specimen number, first occurrence record, collection data, locality, iNaturalist number, and GenBank accession number for specimens of *A. hortensis*, *C. barbara*, *L. cylindracea*, *P. albilabris*, and *X. conspurcata* collected in southern California. Specimen lots are ordered chronologically by collection date. A dash indicates no iNaturalist number or COI sequence for that specimen; an asterisk (*) indicates the collector is not a citizen scientist.

LACM no.	Collector	iNat. no.	Collection date	GenBank COI acc. no.	Collection locality in southern California and first occurrence record
<i>Arion hortensis</i> Férussac, 1819					
Los Angeles County					
179178				MG190382	UCLA campus, near Election Walk, Los Angeles
179180				MG190383	UCLA campus, near Election Walk, Los Angeles
179164			10-Mar-16	MG190384	UCLA campus, near Anderson courtyard, Los Angeles
179162	C. Lee	–		MG190385	UCLA campus, near Anderson courtyard, Los Angeles
179163				MG190386	UCLA campus, at Women's Softball field, Los Angeles
179179				MG190388	Sunset Blvd. at S. Bentley and Greenfield, Los Angeles
179181			13-Mar-16	MG190390	McPherrin Ave. near Garcelon Ave, Monterey Park
180322		4554285	8-Nov-16	–	Bellagio and De Neve Dr., Los Angeles
2017-8.4	J.E. Vendetti*	8712051	2-Nov-17	MG813876	UCLA campus, near Powell Library, Los Angeles
<i>Cochlicella barbara</i> (Linnaeus, 1758)					
Los Angeles County and Orange County					
180088	S. Nakata	–	11-Nov-15	–	Orange Co., near 23701 Moulton Pkwy, Laguna Hills
180085	E. and G. Han	–	1-Feb-16	–	L.A. Co., near Quail and Glenalbyn Dr., Los Angeles
179412			13-May-16	MG195978	L.A. Co., near Quail and Glenalbyn Dr., Los Angeles
180089			11-Nov-16	–	Orange Co., near 23501 Via Mariposa E., Laguna Hills
180133	S. Nakata	4548819	12-Nov-16	–	Orange Co., near 23701 Moulton Pkwy, Laguna Hills
<i>Lauria cylindracea</i> (Da Costa, 1778)					
Los Angeles County and U.S.A.					
179780		2602677	20-Jan-16	KX756234	UCLA campus, near Election Walk, Los Angeles
180086	C. Lee	3862136	11-Aug-16	–	UCLA campus, west of Fowler Museum, Los Angeles
180087		3862135	1-Sep-16	–	UCLA campus, near Election Walk, Los Angeles
2017-8.1	J.E. Vendetti*	8712053	2-Nov-17	MG813887	UCLA campus, at Powell Library, Los Angeles
<i>Pupoides albilabris</i> (C.B. Adams, 1841)					
Riverside County					
179582			20-Feb-17	MG813888	Near Grandview Ave. and Vista Dr., Cathedral City
179582	P. LaFollette*	–	20-Feb-17	MG813889	Near Grandview Ave. and Vista Dr., Cathedral City
<i>Xerotracha conspurcata</i> (Draparnaud, 1801)					
Los Angeles County					
178850	C. McNassor	–	10-Aug-15	–	Between Washburn Rd. and Avenue 63, Pasadena
178920			1-Feb-16	–	Near Quail Dr. and Glenalbyn Dr., Los Angeles
180400	E. and G. Han	2616486	1-Feb-16	KX577716	Near Quail Dr. and Glenalbyn Dr., Los Angeles
178919			2-Feb-16	–	Near Quail Dr. and Glenalbyn Dr., Los Angeles
179352	D. Escobar I. Sanchez	–	16-Mar-16	KX577717	Arroyo Seco Museum Science Magnet, Los Angeles
179361	Lascano family	–	?- Apr-16	–	Near Teresa and Bailey Ave., Rosemead
178921	E. and G. Han	2911675	14-Apr-16	–	Near Quail Dr. and Glenalbyn Dr., Los Angeles
179032	K. Halsey and C. Lee	4467421	1-Nov-16	MG813890	At Solano Ave. and Bouett St., Los Angeles
179351	E. Pogosyan	4936439	12-Jan-17	MG813891	San Gabriel Bldg., Glendale Com. College, Glendale
180083	C. Lee	5140224	19-Feb-17	–	At W. Frontenac and W. Ave. 45, Los Angeles
179606	C. McNassor	–	1-Apr-17	MG813893	Between Washburn Rd. and Avenue 63, Pasadena
2017-4.2	C. Kastely	–	8-Apr-17	–	Near hilltop along Chadwick Circle, Los Angeles
179779	I. Hayden	–	12-Apr-17	MG813892	Near Monrovia High School, Monrovia

step at 94 °C, 40 amplification cycles at 94 °C for 30s (denaturation), 40 °C for 45s (annealing), 72 °C for 60s (extension), with a final extension step at 72 °C for 7 mins. PCR products were verified by gel electrophoresis using a 1%

agarose gel containing ethidium bromide, then purified and sequenced in both directions by Retrogen, Inc. (San Diego, CA) using PCR primers. Resulting chromatograms were inspected for quality, aligned, trimmed of primers in Geneious® version

8.1.6 (Kearse *et al.* 2012), and submitted to GenBank. Specimen collector, collection localities, NHMLA Malacology collection lot numbers and GenBank accession numbers for COI (hereafter abbreviated as acc. no. or acc. nos.) are listed in Table 2.

BLAST and IDS analysis

Specimen COI sequences were compared to those within the NCBI (GenBank) and BOLD (The Barcode of Life Database) databases using NCBI nucleotide BLAST (Basic Local Alignment Search Tool) (Altschul *et al.* 1990) and BOLD IDS (Identification System) (Ratnasingham and Hebert 2007), which report percent similarity or identity scores. GenBank and BOLD databases do not simultaneously share COI sequence data, so search results of both databases are reported herein.

RESULTS

SLIME

As of September 2018, SLIME contributors have generated 9222 photo vouchers of 106 terrestrial gastropod species from southern California, and approximately 1220 citizen scientists have participated in the project. From 2016–2018, annual SLIME-associated bioblitzes hosted on iNaturalist (*e.g.* www.inaturalist.org/projects/el-nino-snailblitz) have each generated over 1100 observations of 45–57 species, including the first U.S. record of *L. cylindracea* (iNat 2602677) and multiple first records for iNaturalist including the native and

imperiled megomphid gastropods, *Glyptostoma newberryanum* (Binney, 1858) (*e.g.* iNat 5113615) and *G. gabrielse* Pilsbry, 1938 (*e.g.* iNat 10235785) (NatureServe 2017). Other commonly observed species by SLIME and SLIME bioblitz contributors between 2015–2018 are *Cochlicopa lubrica* (Müller, 1774), *Cornu aspersum* (Müller, 1774), *Deroceras reticulatum* (Müller, 1774), *Discus rotundatus* (Müller, 1774), *Helminthoglypta traskii* (Newcomb, 1861), *Limacis flavus* Linnaeus, 1758, *Milax gagates* (Draparnaud, 1801), *Otala lactea* (Müller, 1774), *Oxychilus draparnaudi* (Beck, 1837), *Paralaoma servilis* (Shuttleworth, 1852), *Theba pisana* (Müller, 1774), *Vallonia* spp. Risso, 1826, and *Zonitoides arboreus* (Say, 1816) (Stearns 1900, Hanna 1966, Roth and Sadeghian 2006). Approximately 450 specimen lots have been collected as a result of SLIME, which has increased the NHMLA terrestrial gastropod collection by 200% for terrestrial slugs and 5% overall.

Arion hortensis Férussac, 1819 (Fig. 1A, Fig. 2, Table 2)

First record in Los Angeles County, California. The first record of *A. hortensis* in Los Angeles County (iNat 2353797), known to the authors, was made on 1 November 2015 in the city of Monterey Park by C. Lee, a University of California, Los Angeles (UCLA) undergraduate at the time and a prolific iNaturalist user. Subsequently, slugs were collected near this locality and elsewhere in Los Angeles from 2016–2017 (*e.g.* LACM 179179, LACM 179181).

Size and habitat. In a subsample of *A. hortensis* specimens collected from the UCLA campus in 2017 (iNat 8712051, LACM 2017-8.4), average slug length was 16 mm in adults ($N = 12$, $SD \pm 3.0$) and 5.5 mm in juveniles ($N = 2$, $SD \pm 0.7$). At this site,

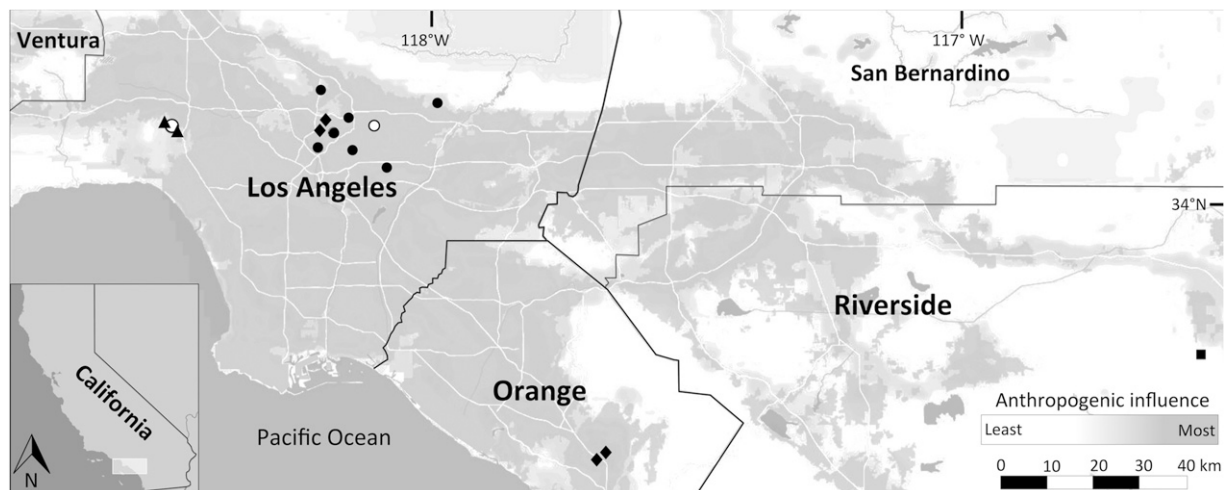


Figure 1. Collection localities of NHMLA specimens (from 2015–2017) of *A. hortensis* (white circle), *C. barbara* (diamond), *L. cylindracea* (triangle), *P. albilabris* (square), and *X. conspurcata* (black dot) in three counties of southern California. Shades of gray indicate the degree of anthropogenic alteration of the land or “human footprint” of Woolmer *et al.* (2008), which incorporates urban development, human population density, and freeways (shown as thin white lines).

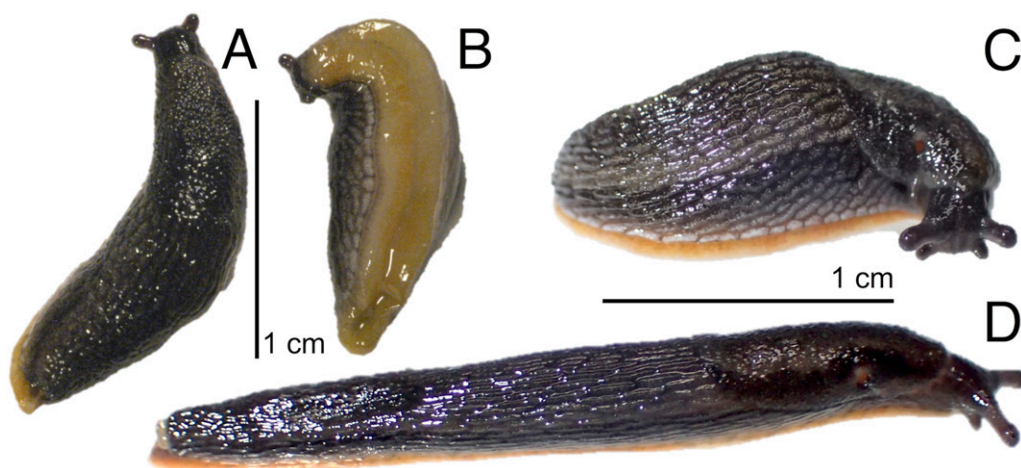


Figure 2. Observations and/or collected specimens of *A. hortensis* from the city of Los Angeles, Los Angeles Co., California, by C. Lee. A–B. from landscaping along Sunset Blvd. between S. Bentley Ave. and Greenfield Ave., 10 March 2016, LACM 179179. C–D. from the UCLA campus, 8 November 2016, iNat 4554285, LACM 180322.

once *A. hortensis* was located, a 45-minute search revealed 15 individuals in leaf litter and landscaped areas adjacent to academic buildings. *Cochlicopa lubrica* (Müller, 1774), *D. rotundatus*, *P. servilis*, and *D. reticulatum* were also found at this site.

Sequence data. NCBI nucleotide BLAST analysis of COI sequences from eight Los Angeles County-collected specimens (acc. no. MG190382–86, MG190388, MG19090, LACM 179162–64, 179178–81) returned a 99–100% identity score with *A. hortensis*, including one specimen collected in Kentucky (acc. no. EU382742, Mc Donnell *et al.* 2008) and 11 unpublished sequences submitted by Dodd *et al.* (2003) and collected in Wales (e.g. acc. nos. AY423681, AY423688) (Symondson, pers. comm.). BOLD IDS analysis returned similar results.

***Cochlicella barbara* (Linnaeus, 1758) (Fig. 1B, Fig. 3, Table 2)**

First record in Los Angeles and Orange counties, California. The first record of *C. barbara* in Los Angeles County, known to the authors, was made on 1 February 2016 (LACM 180085) in the backyard of a home in the Mt. Washington neighborhood of Los Angeles wherein multiple individuals were observed and collected by homeowners, E. and G. Han. Subsequent observations were made by contributors to SLIME on iNaturalist at this locality (e.g. iNat 2903166, 2993738), and by S. Nakata (iNat 10177037) and I. Hayden (iNat 9852531) elsewhere in Los Angeles County in 2015 and 2016. The first record of this species in Orange County was made on 11 November 2015 by S. Nakata from empty shells collected in Laguna Hills (LACM 180088). Species observations contributed to SLIME on iNaturalist also confirm that *C. barbara* remains established in San Diego County (e.g. iNat 9444317, 5656914) (Roth and Hertz 1997).

Size and habitat. In a subsample of *C. barbara* specimens both live and dead-collected in Los Angeles (LACM 179412) and Orange County (LACM 180085, 180088, 180089) average adult shell length was 8.6 mm ($N = 8$, $SD \pm 1.4$) and width was 5.2 mm ($N = 8$, $SD \pm 0.45$). At the Mt. Washington locality, land snails *H. traskii*, *X. conspurcata*, and *O. draparnaudi* were also found.

Sequence data. NCBI nucleotide BLAST analysis of one COI sequence from a Los Angeles-collected specimen (acc. no. MG195978, LACM 179412) returned a

98% identity score with *C. conoidea* collected in Portugal (acc. no. KY818425, Neiber *et al.* 2017), the only other COI sequence identified as *Cochlicella* in GenBank. BOLD IDS analysis returned an approximately 98% similarity score for *C. barbara* with unavailable “Early-Release” sequence accession numbers and locality data.

***Lauria cylindracea* (Da Costa, 1778) (Fig. 1C, Fig. 4, Table 2)**

First record in the United States and Los Angeles County, California. The first record of *L. cylindracea* in the U.S.A., known to the authors, was made by C. Lee on 20 January 2016 on the UCLA campus (iNat 2602677, LACM 179780). Subsequent observations and collection of *L. cylindracea* at and near this site, including adults and juveniles, continued into 2017 (e.g. iNat 3862135, LACM 180086). *Lauria cylindracea* has not been reported from beyond the UCLA campus.

Size and habitat. The average shell length of a subsample of live-collected *L. cylindracea* specimens from the UCLA campus in 2016 (LACM 180087) was 3.8 mm in adults ($N = 8$, $SD \pm 0.27$) and 2.2 mm in juveniles ($N = 5$, $SD \pm 0.19$). At UCLA, *L. cylindracea* was most commonly found between the sidewalk edge and a regularly watered area with sycamore (*Platanus* sp.) leaf litter and native and non-native shrubs. Other co-occurring terrestrial gastropods were *A. hortensis*, *O. draparnaudi*, *D. rotundatus*, *P. servilis*, *Vallonia* spp., and *Z. arboreus*.

Sequence data. NCBI nucleotide BLAST analysis of two COI sequences from specimens collected in Los Angeles (acc. nos. KX756234 and MG813887, LACM 179780, LACM 2017–8.1) returned a 96% identity score with an unpublished *L. cylindracea* sequence submitted by Pokryszko *et al.* (2014)

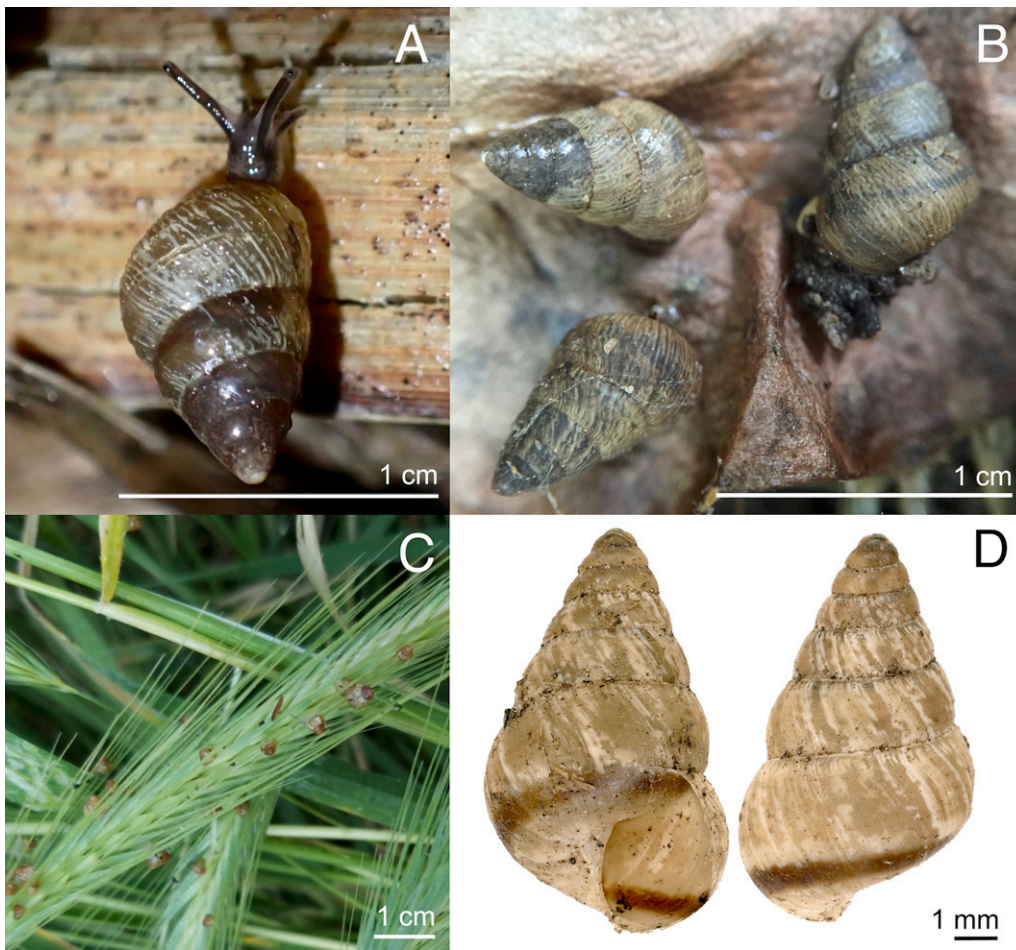


Figure 3. Observations and/or collected specimens of *C. barbara* from Los Angeles, Orange, and San Diego counties, California. A. from Camp Pendleton North, San Diego Co., 10 January 2018 by A. Bairstow, iNat 9444317. B. from a residential backyard, Mt. Washington, Los Angeles Co., 7 May 2016 by E. Han, iNat 3119173. C. from lot beside residential backyard, Mira Mesa, San Diego Co., 29 March 2017 by B. J. Lloyd, iNat 5498858. D. from landscaping along a restaurant perimeter in Laguna Hills, Orange Co., 11 November 2015 by S. Nakata, LACM 180088.

from a specimen collected on the island of Madeira (acc. no. KJ452759) (Cameron, pers. comm.). BOLD IDS analysis returned a 100% similarity score for *L. cylindracea* with unavailable “Early-Release” sequence accession numbers and locality data.

***Pupoides albilabris* (C.B. Adams, 1841) (Fig. 1D, Fig. 5, Table 2)**

First record in Riverside County, California. The only record of *P. albilabris* in California since 1963 and the first in Riverside County, known to the authors, was made on 20 February 2017 in Cathedral City (LACM 179582) in the lawn grass outside the residence of P. LaFollette, an NHMLA Malacology museum associate. Notably, without the attention

and expertise of co-author C. Lee and curation emphasis on terrestrial gastropods because of SLIME, this species would have remained erroneously identified as *L. cylindracea*, with which it shares a pupiform shape and small size (<4 mm).

Size and habitat. In a subsample of *P. albilabris* collected from the Cathedral City locality (LACM 179582), average shell diameter was 3.54 mm in adults ($N = 12$, $SD \pm 0.39$) with no juveniles collected. Co-occurring gastropods include *Gastrocopta pellucida hordeacella* (Pilsbry, 1890), *Hawaiiia minuscula* (Binney, 1840), *Polygyra cereolus* (Mühlfeld, 1816), *Rumina decollata* (Linnaeus, 1758), *Vallonia excentrica* Sterki, 1893, *Vallonia pulchella* (Müller, 1774), and *Vitrea contracta* (Westerlund, 1871).

Sequence data. BOLD IDS analysis of two COI sequences from two specimens collected in Cathedral City (acc. nos. MG813888 and MG813889, LACM 179582) returned a 91.09% similarity score for *P. albilabris* collected in Ontario, Canada in 2015

(FTMW0150-16.COI-5P, FTMW0149-16.COI-5P). NCBI nucleotide BLAST analysis returned an <90% identity score with *Vertigo ovata* Say, 1822 (acc. no. JN941067, Nekola *et al.* 2012) and *Partula similaris* Hartman, 1886 (acc. no. HQ230001, Ó Foighil *et al.* 2011), which have the most similar COI sequence to *P. albilabris* currently within GenBank.

***Xerotracha conspurcata* (Draparnaud, 1801) (Fig. 1E, Fig. 6, Table 2)**

First record in Los Angeles County, California. The first record of *X. conspurcata* in Los Angeles County (LACM 178850), known to the authors, was made on 10 August 2015 in Pasadena by C. McNassor, NHMLA’s former archivist and regular contributor of land snail specimens to the NHMLA

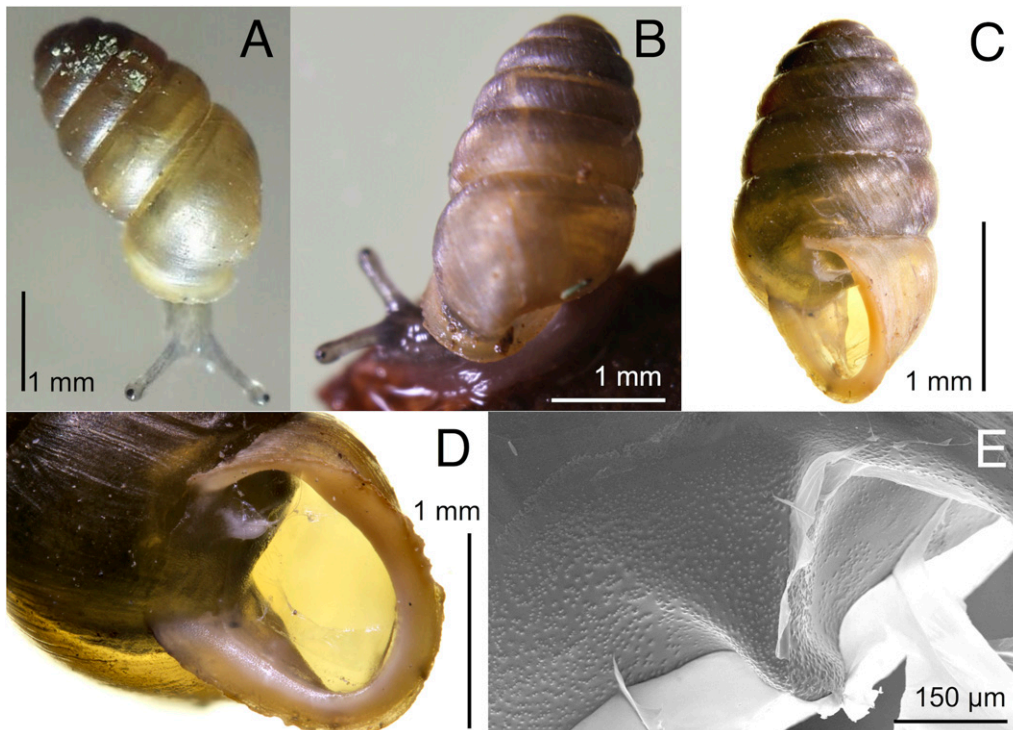


Figure 4. Observations and/or collected specimens of *L. cylindracea* from the UCLA campus, Los Angeles Co., California by C. Lee. A. near Election Walk, 20 January 2016, iNat 2602677, LACM 179780. B–E. in Portolo Plaza, 11 August 2016, iNat 3862135, LACM 180087. C–E. showing angular denticle (C, D) and granular micro-protuberances (E).

Malacology collection. The first record of *X. conspurcata* submitted to SLIME on iNaturalist was made on 23 January 2016 by E. and G. Han (iNat 2616486, LACM 180400) from the yard and external walls of their Los Angeles home. Subsequent observations of *X. conspurcata* were made at various sites in Los Angeles County into 2017, including on the Glendale Community College campus (iNat 4936439, LACM 179351) and in a residential neighborhood in the city of Monrovia (LACM 179779).

Size and habitat. In a subsample of *X. conspurcata* collected from 3 sites in Los Angeles County (LACM 178921, 180083, 180400), average shell diameter was 5.46 mm in adults ($N = 16$, $SD \pm 0.49$) and 3.7 mm in juveniles ($N = 5$, $SD \pm 0.27$). At the Han residence and Glendale Community College campus, once *X. conspurcata* was found, a 10-minute search revealed 10–15 live snails or empty shells. Living snails were found in landscaped and un-maintained habitat, among native and non-native vegetation, and on rocks and structures. At the Han residence, co-occurring gastropods included *H. traskii*, *C. barbara*, *C. lubrica*, *O. draparnaudi*, and *P. servilis*. Live *X. conspurcata* were more often found on vertical surfaces (e.g. building walls) than on foliage or in leaf litter, which likely explains the inadvertent collection of three *X. conspurcata*

individuals in a Malaise trap at the Lascano family's BioSCAN site in Rosemead, California. In the lab, seven live-collected *X. conspurcata* estivated for two months in the absence of moisture, and six resumed crawling and feeding within 10 minutes of soaking their substrate in water. The length of this estivation period is consistent with the findings of Arad *et al.* (1998).

Sequence data. NCBI nucleotide BLAST analysis of COI sequences from six Los Angeles-collected specimens (acc. nos. KX577716-17, MG813890-93, LACM 179032, 179351-52, 179606, 179779, 180400) returned a 98–100% identity score with *X. conspurcata* from Tunisia (acc. no. KU234584, Hausdorf and Boessneck 2016) and *Helicigona lapicida* (Linnaeus, 1758) collected in Germany (acc. no.

AY546280, Steinke *et al.* 2014). This latter record was also returned by BOLD IDS sequence analysis. COI sequences from Los Angeles-collected specimens (e.g. KX577716-17) confirm the suggestion of Groenenberg *et al.* (2011) that the COI sequence identified as *H. lapicida* by Steinke *et al.* (2014) (acc. no. AY546280) in GenBank, is actually from *X. conspurcata*.

DISCUSSION

For NHMs, citizen science that engages the public may facilitate the growth of collections and improve the scope and thoroughness of taxonomic inventories. The SLIME project at NHMLA was designed to engage the public and has facilitated the discovery of five introduced terrestrial gastropod species in the California counties of Los Angeles, Orange, and Riverside. It is also one of few on-going citizen science projects focused on terrestrial gastropods and may be the first to generate county, state, and country first occurrence records of these taxa from a large metropolitan area in North America.

Indices of biodiversity within urban environments rarely focus on terrestrial gastropods although these taxa may be

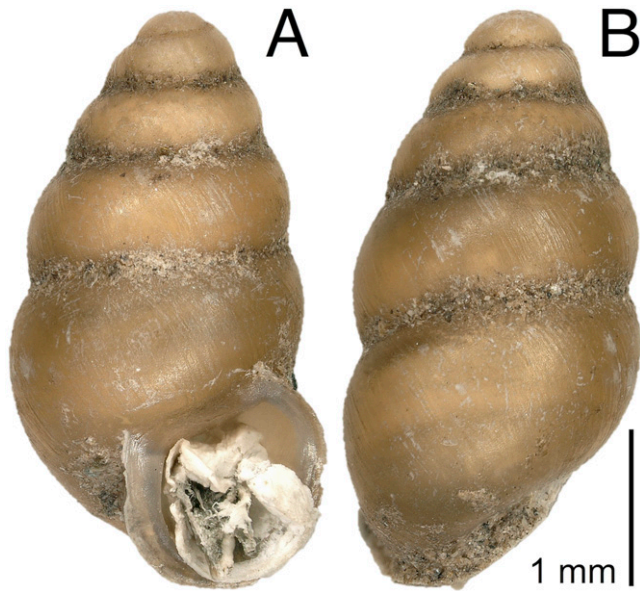


Figure 5. Collected specimens of *P. albilabris* from Riverside Co., California by P. LaFollette. A–B. from a residential lawn in Cathedral City, 20 February 2017, LACM 179582.

easy to find, photograph, and collect. Likewise, evolutionary responses of land snails to urbanization (e.g. reduced gene flow, population fragmentation, adaptation, genetic drift) are largely unstudied (Johnson and Munshi-South 2017), though such research has focused on damselflies (Sato *et al.* 2008), mice (Munshi-South and Kharchenko 2010), bobcats (Serieys *et al.* 2015), and birds (Charmantier *et al.* 2017). While none of the five species documented here by SLIME has become the subject of urban ecology research, citizen scientists' species observations could enable innovative studies on population admixture, responses to habitat fragmentation, and urban-associated life history changes.

Four of the five species discovered in southern California as a result of SLIME (*A. hortensis*, *C. barbara*, *L. cylindracea*, *X. conspurcata*) were documented on multiple occasions at discontinuous sites in populations with juveniles or sub-adults, an indication that these species are breeding and are likely established. Additional species introduction and/or population range expansion may be facilitated by domestic and international trade (e.g. tile, plants), access to water on residential property and parks, and available calcium carbonate from building tile and stucco (Bergey *et al.* 2014). Non-native terrestrial gastropod taxa to anticipate in southern California include the “traveling species” of Smith (1989) and Robinson (1999), and in particular, those that currently thrive in Mediterranean climates. For example, *Microxeromagna lowei* (Potiez and Michaud, 1838) and *Eobania vermiculata* (Müller, 1774) are not known in California at present, but have become

established in Australia (Blackett *et al.* 2016) and South Africa (Herbert 2010). Were these species, or others, to be introduced into southern California, it may be citizen scientists who find them first (Stoeckle 2003).

The morphology, taxonomy, and worldwide distribution (Fig. 7) of the five species discovered in southern California as a result of SLIME are discussed below.

***Arion hortensis* Férussac, 1819 (Fig. 7A)**

Arion hortensis, or *A. (Kobeltia) hortensis*, is a small blue-gray, black, or brown slug of 15 mm to rarely 50 mm in length at maturity with no keel and a distinctly yellow-orange foot sole that produces sticky mucus of the same color(s) (De Winter 1984, Mc Donnell *et al.* 2009, Rowson *et al.* 2014b). Its native range could be western Europe, but where it is truly endemic may be obscured by numerous introductions throughout the region and misidentifications with *Arion distinctus* Mabilie, 1868 and *Arion owenii* Davies, 1979 (Davies 1977, 1979, Iglesias and Speiser 2001, Mc Donnell *et al.* 2008, Rowson *et al.* 2014a, Hatteland *et al.* 2015). Outside of Europe, *A. hortensis* is synanthropic in New Zealand (Barker 1999), Tasmania (Chichester and Getz 1973), South Africa (Herbert and Kilburn 2004, Herbert 2010), and India (Swapna and Reddy 2017), although in the latter three localities slug identity may not have been confirmed by analysis of DNA or reproductive morphology.

In North America, *A. hortensis* has been recorded in Canada and throughout the eastern U.S.A. (Chichester and Getz 1973, McCracken and Selander 1980, Pearce and Bayne 2003), the Great Lakes region (Grimm *et al.* 2009, Steury and Pearce 2014), Kentucky (Mc Donnell *et al.* 2008), the Pacific Northwest, and California (Quick 1952, Severns 2005, Roth and Sadeghian 2006, Mc Donnell *et al.* 2009). *Arion hortensis*, or a member of its species complex, has been intercepted in U.S. agricultural and horticultural imports since at least the 1970s (Girard 1971) and, where pestiferous, *A. hortensis* has damaged sunflower, vegetable, and grain crops (Barker 1999, Iglesias and Speiser 2001, Mc Donnell *et al.* 2009, Thomas *et al.* 2010).

***Cochlicella barbara* (Linnaeus, 1758) (Fig. 7B)**

Cochlicella barbara is a small, xerophilic to mesophilic snail, with a tiny umbilicus and a white/yellow to light brown conical shell of 6–8 slightly convex whorls at maturity (6.5–11 mm in height, 4–6 mm in width) (Welter-Schultes 2012). Its native range spans southern Europe and the Mediterranean region, but it has been introduced to the United Kingdom (Cameron and Killeen 2001, Anderson 2005), Japan (Habe 1980), Israel (Roll *et al.* 2009), Malta (Mifsud *et al.* 2003), Santa Maria in the Azores (Cameron *et al.* 2012), Bermuda (Bieler and Slapcinsky 2000), Madeira (Cook *et al.* 1993), South Africa (Quick 1952 as *Cochlicella ?ventrosa*,

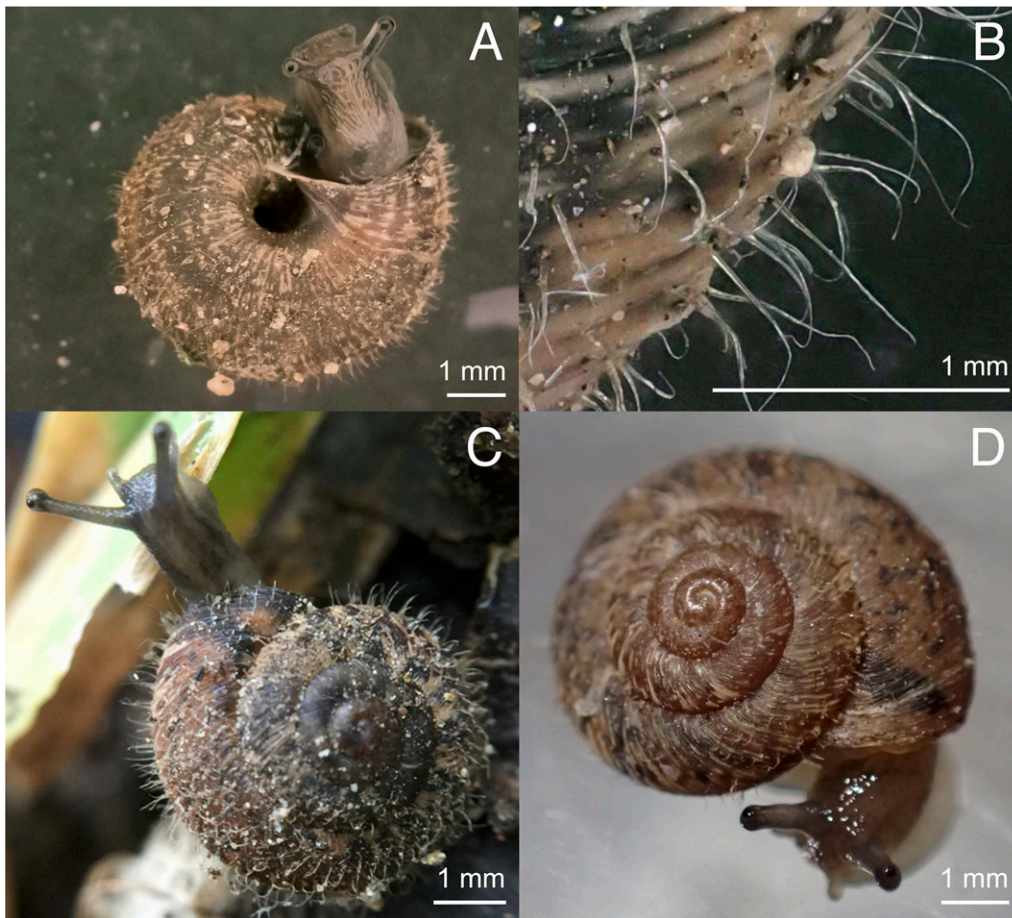


Figure 6. Observations and/or collected specimens of *X. conspurcata* from the city of Los Angeles, Los Angeles Co., California. A–B. from Arroyo Seco Museum Science Magnet campus in Highland Park, 16 March 2016 by D. Escobar and I. Sanchez, LACM 179352. B. showing periostracal hairs. C. from a residential backyard, Mt. Washington, 7 May 2016 by E. Han, iNat 3119164. D. from El Sereno neighborhood, 4 February 2017 by C. Lee, iNat 5056156.

Herbert 2010) where it is considered invasive, New Zealand (Barker 1999), Tasmania (DAFWA 2018), and mainland Australia (Micic *et al.* 2013), where it is an agricultural pest of cereal grain (Baker 1986). In parts of southern Europe where *C. barbara* is native, it lives among ruins, stone walls, and in city parks (Alexandrowicz 2012, Barbato *et al.* 2017).

In North America, *C. barbara* appears to be established only in California, although two previous treatments of introduced and pestiferous terrestrial gastropods did not consider it to be established anywhere in the U.S. (*i.e.* Cowie *et al.* 2009, LaBonte 2009). In 1900, *C. barbara* was documented in residential gardens of Oakland in Alameda County, California (Stearns 1900) and, by the late 2000s it had been reported in Santa Barbara, Santa Clara, Santa Cruz, San Diego, and San Luis Obispo counties (Hanna 1966, Roth and Hertz 1997, Leathers 2015). In 1939, Pilsbry made reference

to *C. barbara* as occurring in South Carolina (as *Cochlicella ventrosa*) based on the published observation of Mazyck (1896), but there are no records of *C. barbara* or its synonyms in subsequent terrestrial gastropod species lists from that region (*e.g.* Hubricht 1971). Notably, *C. barbara* has been intercepted in and on cargo and vehicles in South Carolina, North Carolina, New York, Massachusetts, and Texas, but records of it becoming established in these regions are lacking (Mumford 1965, Adams *et al.* 1990). At U.S. ports including Hawai'i, *C. barbara* snails have been intercepted 608 times between 1985 and 2009 in agricultural and horticultural cargo from Australia, north Africa, and southern Europe (Girard 1971, Michalak and Price 2010). The pest potential of *C. barbara* to grain crops in the U.S. has been recognized by Cowie *et al.* (2009), the California Department of Food and Agriculture (CDFA) (Leathers 2015), and the Cooperative Agricultural Pest Survey (CAPS) program (CAPS Priority Pest List 2019).

***Lauria cylindracea* (Da Costa, 1778) (Fig. 7C)**

Lauria cylindracea is very small, mesophilic snail with an ovoid, medium brown shell of 5–7 moderately convex whorls at maturity (3.5–4.2 mm in height, 2 mm in width). The shell's apertural lip is reflected, slightly thickened, and often bears a single short, angular denticle (Pilsbry and Haas 1922, Grimm *et al.* 2009, Herbert 2010) covered by granular microprotuberances. Its native range is circum-Mediterranean and includes Israel (Mienis 2008), the northern border of the Black Sea (Forsyth 1999, Kantor *et al.* 2009), and continental western Europe (Welter-Schultes 2012). *Lauria cylindracea* has been introduced to the United Kingdom and Ireland (Kerney 1976, Wade *et al.* 2006), islands of the Caribbean and Atlantic (Preece

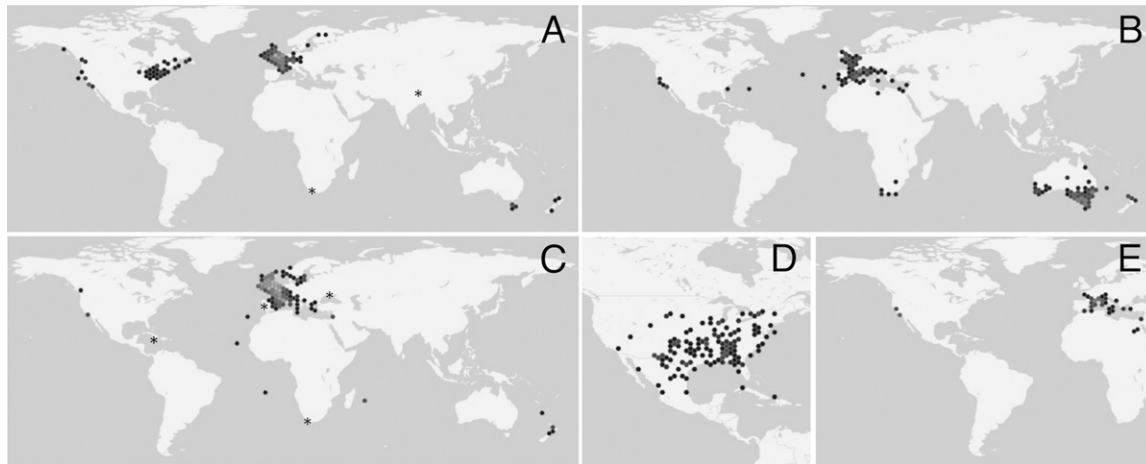


Figure 7. Known global distribution of five terrestrial gastropod species found in southern California, based on GBIF data that include iNaturalist observations (GBIF 2018). Additional localities mentioned in text are marked by an asterisk (*). A. *A. hortensis*. B. *C. barbara*. C. *L. cylindracea*. D. *P. albilabris*. E. *X. conspurcata*.

2001, Rosenberg and Muratov 2006), Réunion (Probst 2001), New Zealand (Willan 1977, Barker 1999), and South Africa, where it is considered invasive (Quick 1952, Herbert 2010). It lives on stone and grass, in crevices of concrete, in gardens and garden waste, and among leaf litter (Pilsbry and Haas 1922, Kerney and Cameron 1979, Heller *et al.* 1997, Georgiev and Stoycheva 2010, Herbert 2010, Welter-Schultes 2012). As a small ground-dwelling detritivore it is not always pestiferous where introduced (Naranjo-García and Castillo-Rodríguez 2017).

The first published observation of *L. cylindracea* in North America was made in 1988 by G. Holm from his residential garden in Richmond, British Columbia, Canada (Holm 1988). Holm hypothesized that *L. cylindracea* snails were accidentally introduced there with a perennial flowering plant *Anemone nemorosa* Linnaeus planted years earlier and brought from Denmark, where *L. cylindracea* is native (Kerney and Cameron 1979). Holm (1988) reports that cuttings of this plant, possibly containing live *L. cylindracea*, were given to friends to establish in their gardens, potentially expanding the range of *L. cylindracea* in the region. Since the late 1980s, *L. cylindracea* has been reported as common in the leaf litter of urban parks in the Canadian cities of Vancouver and Victoria and their suburbs, the Gulf Islands in the Canadian Strait of Georgia, and as far as 85 km east of Vancouver into the Fraser Valley (Holm 1994, Forsyth 1999, Holm 2010, Forsyth and Williston 2012).

***Pupoides albilabris* (C.B. Adams, 1841) (Fig. 7D)**

Pupoides albilabris is a small, xerophilic to mesophilic snail, with a tapering pupiform shell of medium brown color and 5–6.5 strongly convex whorls at maturity (3–5 mm in height, 2 mm in width) (Pilsbry 1948, Fitch and Lokke 1956).

Its native range spans North America from Ontario, Canada, south to Florida, across the American Midwest and Southwest to northern Mexico (Pilsbry 1948, Theler *et al.* 2004, Nekola and Coles 2010). It is not native to California (Pilsbry 1948). *Pupoides albilabris* may be abundant in grasslands and anthropogenically-altered environments such as fallow crop fields, abandoned mining sites, and railroad tracks (Nekola and Coles 2010, Arruda 2014). It has been reported in Cuba, Hispaniola, Puerto Rico, and Bermuda, although at some of these localities it has not been found since initially reported (Pilsbry 1948, Bieler and Slapcinsky 2000). The only published record of *P. albilabris* as introduced in continental North America is of specimens found in California. Hanna (1966) reported its discovery on a lawn of vegetative ground cover in Brawley, Imperial County, California in 1963. Since then there have been no published reports of this species in the state.

***Xerotracha conspurcata* (Draparnaud, 1801) (Fig. 7E)**

Xerotracha conspurcata is a small, xerophilic snail with a light to dark brown lenticular shell of 4–6 whorls at maturity (5–6 mm in diameter) and numerous tiny periostracal hairs, especially in sub-adults. Its modern range is circum-Mediterranean, but this distribution may be a consequence of accidental human introduction during antiquity from its native range on the Iberian Peninsula (Welter-Schultes 2008). Human-facilitated introduction of *X. conspurcata* continues today from western Europe in exported produce (Herbert 2010, Vaisman and Mienis 2012), tile, and seeds (Robinson 1999). At U.S. ports including Hawai‘i, *X. conspurcata* individuals were intercepted 4,425 times between 1985 and 2009 in cargo from 31 countries including Italy, Spain, France, Greece, Turkey, and Israel (Michalak and Price 2010). In

regions of Greece and Italy with climate similar to that of southern California, *X. conspurcata* is widespread and synanthropic, living in disturbed lots and parks (Georgiev and Stoycheva 2010, Barbato *et al.* 2017). In Rome, *X. conspurcata* is one of the most common snails living among ruins of the Roman Forum and Colosseum (Alexandrowicz 2012).

The only reported populations of *X. conspurcata* in North America are from California. The California Academy of Sciences invertebrate zoology collection houses specimens that were collected in 1996 from Contra Costa County during a CDFA inspection of imported slate (Gill 1996). This discovery led to observations of live *X. conspurcata* snails where the slate shipment had been distributed: San Mateo, Alameda, and Sonoma counties (Gill 1996, Roth and Sadeghian 2006, Cowie *et al.* 2009, Michalak and Price 2010). In 2015, CDFA did not consider *X. conspurcata* to be established in California, and rated its pest potential as “B”, denoting a high dispersal ability but low potential for agricultural or environmental impact (Leathers 2015).

ACKNOWLEDGEMENTS

The authors are grateful to NHMLA’s Lindsey Groves and Maria Wong for specimen curation in Malacology, the Community Science Program for promoting SLIME, Greg Pauly and Chris Thacker in Herpetology and Ichthyology for molecular laboratory space, Lisa Gonzalez in Entomology for providing *X. conspurcata* from the Lascano family BioSCAN site, Giar Ann Kung in Entomology for assistance with SEM and Keyence microscopy, and the Urban Nature Research Center (UNRC) for funding and helpful feedback on this paper. We also thank Ms. Griffith and her 2016 ASMSM 8th grade class for surveying their school campus for snails and slugs, the citizen scientists and iNaturalist users R. J. Adams, J. Bailey, J. A. Caballero, C. Hayden, S. Hewitt, T. Lawson, P. Liff-Grieff, D. Loarie, S. Loarie, T. Rahn, and J. Rycenga for valuable species observations and identifications, and Patrick Krug at California State University, Los Angeles for additional laboratory space. We are also grateful to Barry Roth for additional specimen identifications and Robert Cameron at the University of Sheffield and William Symondson at Cardiff University for providing locality data for unpublished GenBank sequences. The comments and suggestions of Elizabeth Shea, Tim Pearce, Wallace Meyer, and two anonymous reviewers greatly improved this manuscript. Importantly, these first occurrence records would not have been possible without the effort and enthusiasm of the citizen science collaborators who participated in the SLIME project: thank you for your diligent documentation of biodiversity. Finally, we express our gratitude to Cathy McNassor, in memoriam, who contributed terrestrial gastropod specimens to NHMLA’s

Malacology collection and was a dedicated staff member at NHMLA and the La Brea Tar Pits and Museum for more than thirty years.

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Submitted: 6 March 2018; **accepted:** 10 September 2018;
final revisions received: 10 October 2018