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First records of four exotic slugs in Argentina

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Abstract: This paper reports for the first time the occurrence of four exotic terrestrial slug species in Argentina: *Lehmannia valentiana* (Férussac, 1823) (Limacidae), *Deroceras invadens* Reise *et al.* 2011 (Agriolimacidae), *Arion intermedius* Normand, 1852 (Arionidae) and *Meghimatium pictum* (Stolyczka, 1873) (Philomycidae). The study is based on specimens deposited in museums in Argentina. Both the morphologic characteristics and the mitochondrial cytochrome oxidase I gene sequences were used to identify the exotic species. Phylogenetic analyses were also carried out in order to explore the location of their origins. *Lehmannia valentiana* had the oldest records and has been widely distributed in Argentina. *Deroceras invadens* and *A. intermedius* were found to be restricted to the southern portion of the country. *Meghimatium pictum* was recorded in the northwest and northeast Argentina, and the DNA sequences analyzed from this species were more closely related to specimens from the west of the Strait of Taiwan. A determination of the origin of the other species was impossible because either the sequences analyzed grouped with samples from different geographical origins or only few sequences were available for comparison. In view of the invasive potential of these slug species, the present work provides new and potentially useful DNA sequence data obtained from morphologically-confirmed specimens. Information provided from these analyses should assist in making a rapid identification of these exotic slugs by nonspecialists and governmental authorities who are responsible for managing and controlling the presence of exotic species.

Key words: *Lehmannia valentiana*, *Deroceras invadens*, *Arion intermedius*, *Meghimatium pictum*, molecular phylogenetic analyses

Biologic invasions, together with climate change and habitat fragmentation, constitute one of the most serious threats affecting the maintenance of global biodiversity (Vitousek *et al.* 1996, Nentwig 2007). Bioinvasions have mostly caused disruptive effects on native species, alteration of ecological processes, changes in natural systems, and economic and human-health problems (Mooney *et al.* 2005). In South America, for example, certain invasive mollusk species – *e.g.*, the continental bivalve *Limnoperna fortunei* (Dunker, 1857), the marine gastropod *Rapana venosa* (Valenciennes, 1846) and the terrestrial snail *Achatina (Lissachatina) fulica* Bowdich, 1822 – have been reported to reduce biodiversity (Darrigran and Damborenea 2006, Giberto *et al.* 2006, Thiengo *et al.* 2007). Some introductions have been deliberate (*e.g.*, *A. fulica*), while others were unintentional (*e.g.*, *L. fortunei*), with the latter being the most common case in point (Cowie and Robinson 2003).

Twenty-three exotic terrestrial gastropod species have been reported in Argentina (Parent and Miquel 1999, Miquel *et al.* 2007, Rumi *et al.* 2010, Gutiérrez Gregoric *et al.* 2011).

Six of these species are slugs: *Limacus flavus* Linnaeus, 1758, *Limax maximus* Linnaeus, 1758, *Deroceras agreste* (Linnaeus, 1758), *Deroceras laeve* (Müller, 1774), *Deroceras reticulatum* (Müller, 1774), and *Milax gagates* (Drapaurnaud, 1801) (Rumi *et al.* 2010). Because of the high intraspecific morphologic variability in slugs, molecular studies have proven to be extremely useful for species identification (McDonnell *et al.* 2008, 2011). Phylogenetic studies conducted on several species have, moreover, enabled a more complete understanding of the history of the invasions and an establishment of the origins of the invaders along with the pathways of their introduction (*e.g.*, Facon *et al.* 2003). These molecular studies, combined with anatomical taxonomic identification and an understanding of those pathways, constitute crucial information for enabling governmental entities to adjust their strategies for controlling invading species (Cowie and Robinson 2003, Lizarralde *et al.* 2008).

The aim of this report is to document for the first time in Argentina the presence of four alien terrestrial slugs from

the families Limacidae, Agriolimacidae, Arionidae and Philomycidae, on the basis of morphologic and molecular-genetic identifications and, wherever possible, to make inferences about the origins of those species and the possible consequences of their introductions.

MATERIALS AND METHODS

Specimen collections and morphology-based identifications

Malacological collections from the Museo de La Plata (MLP), the Museo Argentino de Ciencias Naturales (MACN-In), the Instituto Fundación Miguel Lillo (IFML), and the United States

Department of Agriculture (USDA) were surveyed (Table 1). The specimens chosen (Limacidae $N = 43$; Agriolimacidae $N = 62$; Arionidae $N = 14$; Philomycidae $N = 12$) were dissected under a stereomicroscope and identified mainly on the basis of the reproductive system and/or the external morphology. Adult specimens, properly relaxed, were measured to establish a length range.

DNA extraction, polymerase chain reaction (PCR) amplification, and genetic sequencing

Total DNA was extracted from 2 mm³ samples from the foot of each dissected slug. The tissue was rinsed in distilled

Table 1. Material examined from slugs from Argentina. **IFML**, Instituto Fundación Miguel Lillo; **MACN-In**, Museo Argentino de Ciencias Naturales; **MLP**, Museo de La Plata; **N**, number of specimens; **USDA**, United States Department of Agriculture.

Family	Province	Site	Date	Collection	<i>N</i>	Coordinates
Limacidae	Tucumán	San Miquel	1962	IFML 604	28	26°49'S, 65°12'W
	Chubut	Lago Puelo Nat. Park	2003	IFML 14431	4	42°05'S, 71°36'W
	Buenos Aires	Sierras Bayas	1924	MACN-In 14602	5	36°56'S, 60°09'W
		La Plata	2012	MLP 13636	8	34°55'S, 57°55'W
	Río Negro	El Bolsón	2004	MACN-In 36158	1	51°58'S, 71°32'W
	Neuquén	Neuquén	2004	MACN-In 36162	1	38°56'S, 68°03'W
Agriolimacidae	Río Negro	El Bolsón	2004	MACN-In 36157	2	51°58'S, 71°32'W
				MACN-In 36158/1		
		Dina Huapi	2004	MACN-In 36167	4	41°05'S, 71°10'W
				MACN-In 36168/1		
		San Carlos de Bariloche	2004	MACN-In 36173	33	41°08'S, 71°18'W
				MACN-In 36179–80		
				MACN-In 36182–83		
				MACN-In 36186/2		
				MACN-In 36188–92		
			Cerro Otto	2004	MACN-In 36177	4
		Nahuel Malal	2004	MACN-In 36176	2	41°06'S, 71°26'W
		Manantial	2004	MACN-In 36181/1	2	40°40'S, 71°37'W
	Neuquén	Neuquén	2004	MACN-In 36162	1	38°56'S, 68°03'W
		Isla Victoria	2004	MACN-In 36164	12	40°58'S, 71°31'W
		Villa La Angostura	2004	MACN-In 36169	4	40°45'S, 71°38'W
		Los Alerces Nat. Park	2010	MLP 13405	12	42°36'S, 71°38'W
Arionidae	Chubut			IFML 15569		
		Los Alerces Nat. Park	2003	MLP 13404	5	42°44'S, 71°44'W
				IFML 15463		
		Lago Puelo Nat. Park	2003	IFML 15449	13	42°05'S, 71°36'W
				IFML 15566		
		Lago Puelo Nat. Park	2004	MACN-In 36184	2	42°05'S, 71°37'W
	Río Negro	Guillermo lake	2010	IFML 15568 A	3	41°21'S, 71°29'W
San Carlos de Bariloche		2004	MACN-In 36170	4	41°05'S, 71°27'W	
				MACN-In 36186/1		
Philomycidae	Misiones	Iguazú Nat. Park	2009	MLP 13402	3	25°41'S, 54°27'W
		Tabay Fall	2009	MLP 13403	5	27°00'S, 55°11'W
		Puerto Iguazú	2011	IFMLP 15570 A	1	25°35'S, 54°34'W
	Tucumán	San Miguel	1999	IFML 15571 A	1	26°48'S, 65°17'W
	Brazil	Foz do Iguazu	2011	USDA 110442	2	25°34'S, 54°34'W

water, ground in 100 mM EDTA and 20 mM Tris, and digested overnight in CTAB buffer containing proteinase K. DNA was purified by a threefold extraction with chloroform-isoamyl alcohol (24:1) followed by precipitation with isopropanol. The DNA was then resuspended in TE buffer. A 655 bp fragment of the gene encoding the mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified by means of the primers of Folmer *et al.* (1994).

Amplification was performed in a final volume of 50 µl containing: 50–100 ng of template DNA, 0.1 µM of each primer, 1X PCR buffer, 50 µM dNTPs, 2.5 mM MgCl₂, and 1.2 U Platinum *Taq* polymerase (Invitrogen, Brazil). The thermocycling sequence consisted of 3 min at 94 °C; 5 cycles of 30 s at 94 °C; 40 s at 45 °C; 1 min at 72 °C; followed by 35 cycles of 30 s at 94 °C; 40 s at 51 °C; 1 min at 72 °C; with a final extension for 10 min at 72 °C. After purification of the PCR products by electrophoresis in 1.5% (w/v) agarose gels through the use of a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Orange, California), both DNA strands were sequenced (Macrogen Inc., Seoul, Korea). The resulting

sequences were trimmed to remove the primers, and the consensus sequences of the individuals were compared to reference sequences in GenBank through the use of the BLASTN algorithm (Altschul *et al.* 1990) to identify similarities.

Phylogenetic analyses

Phylogenetic analyses were conducted in order to confirm the morphology-based identification of the specimens found in Argentina and to make inferences on the source location whenever that identification was possible. For the Agriolimacidae we used the COI sequences of almost all the species represented in Reise *et al.* (2011) (Table 2). For the Arionidae, we analyzed the COI sequences available in GenBank mainly for the *Arion* Férussac, 1819 species, those being reported in the literature as either invasive or potentially invasive (McDonnell *et al.* 2009, Thomas *et al.* 2010) (Table 3). For the Philomycidae, we conducted the phylogenetic analyses with the COI sequences for only *Meghimatium pictum* (Stolyczka, 1873) since we counted on a large number of available sequences for that species from various locations

Table 2. Information on the specimens used in the phylogenetic reconstruction of *Deroceras* species. *GenBank unpublished sequences: the sequence author and submission year are indicated.

Taxon	GenBank #	Country	Reference
Outgroup			
<i>Arion distinctus</i> Mabile, 1868	EF128218	Taiwan	Tsai and Wu 2008
<i>Arion rufus</i> (Linnaeus, 1758)	FJ481178	-	Tsai 2008*
<i>Pallifera dorsalis</i> (A. Binney, 1842)	FJ896618	U.S.A.	Tsai <i>et al.</i> 2011
<i>Philomycus carolinianus</i> (Bosc, 1802)	EF128221	U.S.A.	Tsai <i>et al.</i> 2011
<i>Megapallifera ragsdalei</i> (Webb, 1950)	EF128220	U.S.A.	Tsai and Wu 2008
Ingroup			
<i>Deroceras golcheri</i> (Altena, 1962)	JN248291–293	Malta	Reise <i>et al.</i> 2011
<i>Deroceras invadens</i>	FJ358222	South Africa	Reise <i>et al.</i> 2011
	JN248295	Germany	Reise <i>et al.</i> 2011
	JN248296	United Kingdom	Reise <i>et al.</i> 2011
	JN248297	Canada	Reise <i>et al.</i> 2011
	JN248298–300	Italy	Reise <i>et al.</i> 2011
	JN248301–302	Germany	Reise <i>et al.</i> 2011
	JN248303	United Kingdom	Reise <i>et al.</i> 2011
	JN248314	France	Reise <i>et al.</i> 2011
	JN248315	U.S.A.	Reise <i>et al.</i> 2011
	JQ743070	Argentina	This work
<i>Deroceras laeve</i>	AF239733	U.S.A.	Reise <i>et al.</i> 2011
	EF128217	Taiwan	Tsai and Wu 2008
	HM584699	-	Reise <i>et al.</i> 2011
<i>Deroceras panormitanum</i>	JN248304–306	Italy	Reise <i>et al.</i> 2011
	JN248307–311	Malta	Reise <i>et al.</i> 2011
	JN248312–313	Italy	Reise <i>et al.</i> 2011
<i>Deroceras reticulatum</i>	AF239734	U.S.A.	Reise <i>et al.</i> 2011
	AM259702–703	United Kingdom	Reise <i>et al.</i> 2011
	FJ481179	-	Reise <i>et al.</i> 2011

Table 3. Information on the specimens used in the phylogenetic reconstruction of *Arion* species. *GenBank unpublished sequences: the sequence author and submission year are indicated.

Taxon	GenBank #	Country	Reference
Outgroup			
<i>Deroceras leave</i>	EF128217	Taiwan	Tsai and Wu 2008
<i>Deroceras reticulatum</i>	FJ481179	-	Reise <i>et al.</i> 2011
<i>Limacus flavus</i>	FJ481181	-	Tsai 2008*
<i>Megapallifera ragsdalei</i>	EF128220	U.S.A.	Tsai and Wu 2008
<i>Philomycus carolinianus</i>	EF128221	U.S.A.	Tsai and Wu 2008
Ingroup			
<i>Arion circumscriptus</i> Johnston, 1828	AY094600	Lithuania	Soroka 2002*
	AY987872	Ireland	Davison <i>et al.</i> 2009
	AY987873	Belgium	Davison <i>et al.</i> 2009
	DQ647392	Lithuania	Soroka 2006*
<i>Arion distinctus</i>	AY094599	Poland	Soroka and Skujienė 2011
	AY987874	Belgium	Pinceel <i>et al.</i> 2005*
	AY987876	Belgium	Pinceel <i>et al.</i> 2005*
	DQ647393	Poland	Soroka and Skujienė 2011
<i>Arion fasciatus</i> (Nilsson, 1823)	AF239735	U.S.A.	Remigio and Hebert 2003
	AY094598	Lithuania	Soroka 2002*
	AY987877	Germany	Davison <i>et al.</i> 2009
	AY987878	Austria	Davison <i>et al.</i> 2009
<i>Arion fuscus</i> (Müller, 1774)	AY094597	Lithuania	Soroka and Skujienė 2011
	AY987885	Poland	McDonnell <i>et al.</i> 2011
	AY987886	Bulgaria	McDonnell <i>et al.</i> 2011
	DQ647391	Lithuania	Soroka 2006*
<i>Arion hortensis</i> Férussac, 1819	AY423670	-	Davison <i>et al.</i> 2009
	AY423688	-	Dodd <i>et al.</i> 2003*
	AY987889	United Kingdom	Davison <i>et al.</i> 2009
	EU382742	U.S.A.	McDonnell <i>et al.</i> 2008
<i>Arion intermedius</i>	AM259724	United Kingdom	McClymont 2006*
	AY987891	Belgium	Davison <i>et al.</i> 2009
	EU382756	U.S.A.	McDonnell <i>et al.</i> 2008
	JQ743069	Argentina	This work
<i>Arion lusitanicus</i> Mabille, 1868	AY987894	Belgium	Davison <i>et al.</i> 2009
	EF520640	Poland	Soroka <i>et al.</i> 2009
	EF535149	Poland	Soroka <i>et al.</i> 2009
	EU734828	Belgium	Soroka and Kałuski 2011
<i>Arion rufus</i>	EF520644–647	Poland	Soroka <i>et al.</i> 2009
<i>Arion owenii</i> Davies, 1979	AY423702	-	Davison <i>et al.</i> 2009
	AY423703	-	Dodd <i>et al.</i> 2003*
	AY987897	United Kingdom	Pinceel <i>et al.</i> 2005*
	AY987898	United Kingdom	Davison <i>et al.</i> 2009
<i>Arion silvaticus</i> Lohmander, 1937	AF513018	Lithuania	Góbbeler and Klussmann-Kolb 2010
	AY987917–918	Belgium	Davison <i>et al.</i> 2009
<i>Arion subfuscus</i> (Draparnaud, 1805)	AY987905	Belgium	McDonnell <i>et al.</i> 2011
	AY987914	France	McDonnell <i>et al.</i> 2011
	AY987916	France	McDonnell <i>et al.</i> 2011
	GU249583	U.S.A.	McDonnell <i>et al.</i> 2011

cited in Gomes *et al.* (2011) and Tsai *et al.* (2011) (Table 4). Finally, phylogenetic analyses were not possible for the Limacidae because few sequences were available in GenBank, with the molecular identification of this species based only on the BLASTN algorithm.

In all instances, the phylogenetic analyses were carried out as follows: the sequence alignment was performed with the Clustal X 2.0.12 software (Larkin *et al.* 2007), optimized by visual inspection, and edited with a word processor. The total lengths of the matrices analyzed were 534 bp for

the Limacidae, 552 bp for the Arionidae, and 654 bp for the Philomycidae. The data were subjected to four different phylogenetic analyses by the methods of Neighbor-Joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). The NJ analysis was conducted with MEGA 5.05 software (Tamura *et al.* 2011) through the use of the maximum-composite-likelihood option for computing evolutionary distances (Tamura *et al.* 2004). The MP analysis was carried out with the PAUP*4.0b10 software (Swofford 2002), through the use of heuristic search, characters equally

Table 4. Information on the specimens used in the phylogenetic reconstruction of *Meghimatium pictum*. **CMS-DPE**, Superintendência de Controle de Endemias do Estado de São Paulo, Brazil; **ESRI-MOL**, Endemic Species Research Institute, Taiwan; **MLP**, Museo de La Plata, Argentina; **MZSP**, Museu de Zoologia da Universidade de São Paulo, Brazil; **USDA**, United States Department of Agriculture, U.S.A. *GenBank unpublished sequences: the sequence author and submission year are indicated.

Codification	GenBank #	Country	Location	Reference
Outgroup				
<i>Arion distinctus</i>	EF128218	Taiwan	Wuling farm, Taichung	Tsai and Wu 2008
<i>Arion rufus</i>	FJ481178	-	-	Tsai 2008*
<i>Deroceras laeve</i>	EF128217	Taiwan	Guansi, Hsinchu	Tsai and Wu 2008
<i>Deroceras reticulatum</i>	FJ481179	-	-	Reise <i>et al.</i> 2011
<i>Limacus flavus</i>	FJ481181	-	-	Tsai 2008*
<i>Megapallifera ragsdalei</i>	EF128220	U.S.A.	Seatey, Alaska	Tsai and Wu 2008
<i>Pallifera dorsalis</i>	FJ896618	U.S.A.	-	Tsai <i>et al.</i> 2011
<i>Philomycus carolinianus</i>	EF128221	U.S.A.	Carter, Tennessee	Tsai and Wu 2008
Ingroup				
<i>Meghimatium pictum</i>				
ARP1 (MLP 13403/1)	JQ712575	Argentina	Tabay Fall, Misiones	This work
ARP2 (MLP 13403/2)	JQ712574	Argentina	Tabay Fall, Misiones	This work
BRP1 (CMS-DPE-95)	HM233929	Brazil	Ribeirão Pires, São Paulo	Gomes <i>et al.</i> 2011
BRP2 (MZSP 93842)	HM233928	Brazil	Curitiba, Paraná	Gomes <i>et al.</i> 2011
BRP3 (MZSP 93836)	HM233930	Brazil	Palhoça, Santa Catarina	Gomes <i>et al.</i> 2011
BRP4 (USDA 110442)	JQ712572	Brazil	Foz do Iguaçu, Paraná	This work
BRP5 (USDA 110442)	JQ712573	Brazil	Foz do Iguaçu, Paraná	This work
CNP1 (ESRI-MOL-07-0123)	FJ896666	China	Linan, Zhejiang	Tsai <i>et al.</i> 2011
CNP2 (MZSP 93847)	HM233931	China	Zhongcun, Guangzhou	Gomes <i>et al.</i> 2011
TLP1 (ESRI-MOL-05-0024)	FJ896667	Thailand	Bangkok	Tsai <i>et al.</i> 2011
TWP1 (ESRI-MOL-08-0565)	FJ896651	Taiwan	Wushikeng, Taichung	Tsai <i>et al.</i> 2011
TWP2 (ESRI-MOL-07-0117)	FJ896652	Taiwan	Yousheng river, Taichung	Tsai <i>et al.</i> 2011
TWP3 (ESRI-MOL-08-0264)	FJ896653	Taiwan	Lugu, Nantou	Tsai <i>et al.</i> 2011
TWP4 (ESRI-MOL-08-0566)	FJ896654	Taiwan	Wanluan, Pingtung	Tsai <i>et al.</i> 2011
TWP5 (ESRI-MOL-08-0138)	FJ896655	Taiwan	Litao, Taitung	Tsai <i>et al.</i> 2011
TWP6 (ESRI-MOL-06-0295)	FJ896656	Taiwan	Lanyu, Taitung	Tsai <i>et al.</i> 2011
TWP7 (ESRI-MOL-99-0001)	FJ896657	Taiwan	Ruisui, Hualien	Tsai <i>et al.</i> 2011
TWP8 (ESRI-MOL-02-0003)	FJ896658	Taiwan	Meifeng farm, Nantou	Tsai <i>et al.</i> 2011
TWP9 (ESRI-MOL-08-0567)	FJ896659	Taiwan	Jiufen, Rueifang, Taipei	Tsai <i>et al.</i> 2011
TWP10 (ESRI-MOL-01-0002)	FJ896660	Taiwan	Taman, Wulai, Taipei	Tsai <i>et al.</i> 2011
TWP11 (ESRI-MOL-07-0121)	FJ896661	Taiwan	Kinhu, Kinmen	Tsai <i>et al.</i> 2011
TWP12 (ESRI-MOL-07-0122)	FJ896662	Taiwan	Lieyu	Tsai <i>et al.</i> 2011
TWP13 (ESRI-MOL-07-0125)	FJ896663	Taiwan	Beigan, Matsu	Tsai <i>et al.</i> 2011
TWP14 (ESRI-MOL-06-0296)	FJ896664	Taiwan	Nangan, Matsu	Tsai <i>et al.</i> 2011
TWP15 (ESRI-MOL-07-0124)	FJ896665	Taiwan	Dongyin, Matsu	Tsai <i>et al.</i> 2011

weighted, tree bisection and reconnection branch-swapping and 10 random stepwise additions. The ML inference was performed by means of the PhyML program (Guindon and Gascuel 2003) available at the public Phylemon2 webserver (<http://phylemon.bioinfo.cipf.es>; Sánchez *et al.* 2011). The optimal model of nucleotide substitution was evaluated by the likelihood-ratio test and selected by means of the corrected Akaike Information Criterion with the Jmodeltest 0.1.1 software (Posada 2008). The HKY+I+G (for Limacidae), the TIM2+I+G (for Arionidae), and the GTR+I+G (for Phylomicidae) substitution models were used as evolutionary paradigms. The statistical support for the resulting phylogenies were assessed by bootstrapping with either 1,000 (NJ, MP) or 100 (ML) replicates (Felsenstein 1985). The BI was carried out with the Mr. Bayes 3.1.2 software (Ronquist and Huelsenbeck 2003). Two runs were performed simultaneously with 4 Markov chains that went for 1,000,000 generations, sampling every 100 generations. The first 10,000 generations of each run were discarded as burnin, and the remaining 18,000 trees were used to estimate posterior probabilities. All the trees were edited with the TreeGraph 2 software (Stöver and Müller 2010).

RESULTS

Family LIMACIDAE Lamarck, 1801

The adult specimens had a mean total length of 27.21 mm ($N = 13$; $SD = 5.24$; range = 20.7–39.5 mm), with an ill-defined keel at the hind end. The external morphology and reproductive system of the dissected adult specimens were consistent with descriptions by Castillejos and Garrido (1996) for *Lehmannia valentiana* (Férussac, 1823). Specimens from IFML 14431 were identified only by external morphology. Body color of slugs varied from chestnut or auburn to yellowish gray, with two or three black longitudinal bands on the mantle shield and only two on the rest of the body, finalizing in the posterior end. The central band on the mantle shield was diffuse in some cases. Pneumostome was on the right posterior part of mantle. The specimens had been found in urban and protected zones (*e.g.*, Lago Puelo National Park). *Lehmannia valentiana* has a widespread distribution in Argentina (Fig. 1), and specimens were recorded together with other native and exotic gastropods (*e.g.*, *Deroceras reticulatum* and *Limacus flavus* in La Plata city). The molecular data confirmed the morphology-based identification. The BLASTN search results, with the obtained partial COI sequence as the query sequence (Gen Bank JX117876, 655 bp), showed top-ranking scores and a 99% sequence identity with the only two 554 bp reference COI sequences available in the GenBank nucleotide database (AM259710 and AM259711).

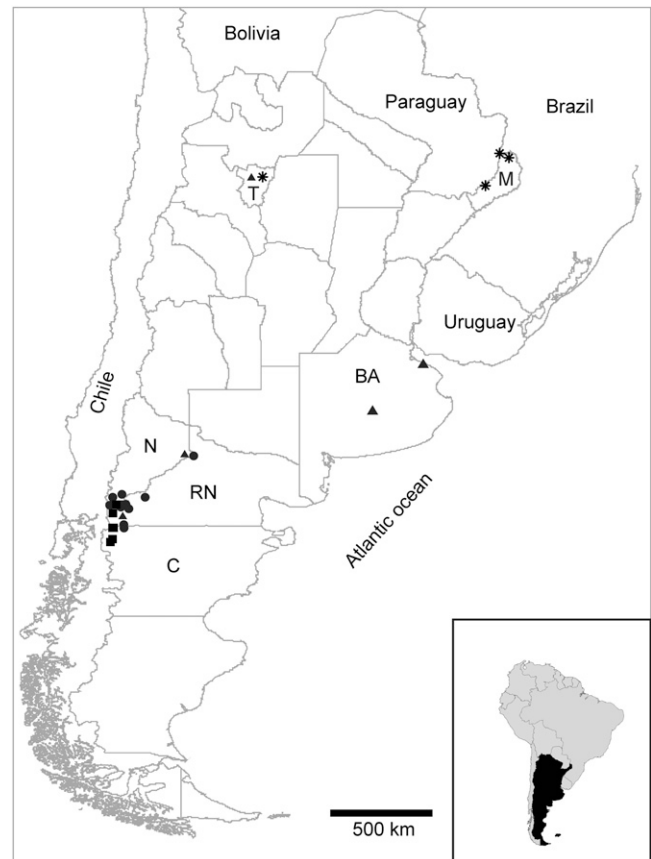


Figure 1. Distribution of new exotic slugs recorded in Argentina. ▲: *Lehmannia valentiana*; ●: *Deroceras invadens*; ■: *Arion intermedius*; *: *Meghimatium pictum*. Provinces: BA, Buenos Aires; C, Chubut; M, Misiones; N, Neuquén; RN, Río Negro; T, Tucumán.

Family AGRIOLIMACIDAE Wagner, 1935

The length of the adult specimens was in general around 12.74 mm ($N = 51$; $SD = 1.38$; range = 9.5–16.4 mm). The external morphology and reproductive system of the dissected specimens ($N = 62$) were consistent with descriptions by Reise *et al.* (2011) for *Deroceras invadens* Reise *et al.*, 2011. This species has been recorded in both urban and protected areas (*e.g.*, Nahuel Huapi and Arrayanes National Parks) (Fig. 1). The identification of the Agriolimacidae specimen analyzed from Argentina based on molecular analysis confirmed its specific identity as *D. invadens*. The trees obtained by different methods indicated a similar topology that was in agreement with Reise *et al.* (2011; Fig. 2 cf. the MP and BI trees). In all instances, this specimen from Argentina belonged to the *D. invadens* clade, which assignment was highly supported (NJ = 100, MP = 98, ML = 100, BI = 1), and was furthermore classified in a subgroup inside this clade (NJ = 87, MP = 93, ML = 84, BI = 0.85) one that included sequences from several parts of the world (*i.e.*, Canada, England, France, Germany, South Africa and U.S.A).

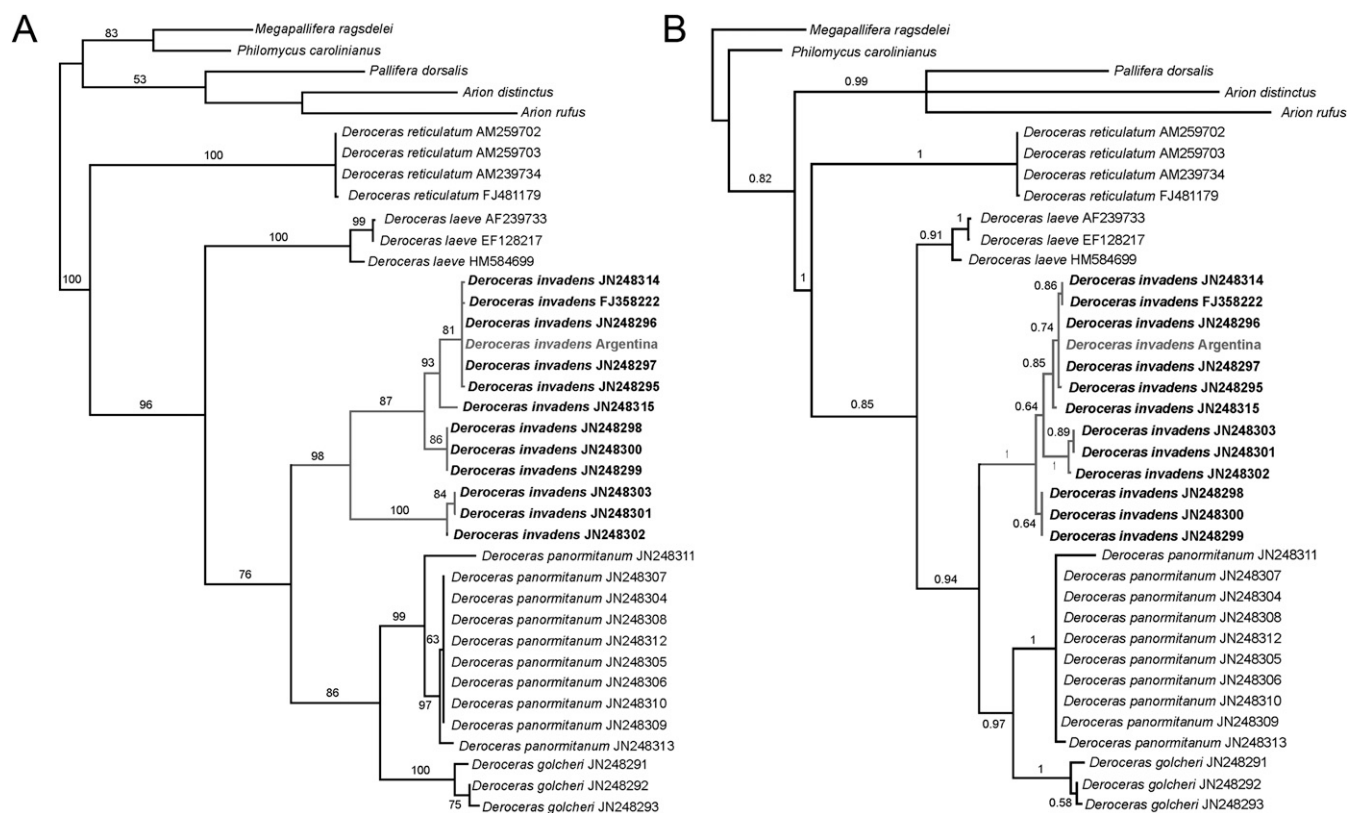


Figure 2. Phylogenetic trees of several *Deroceras* species based on 534 nucleotides of the partial COI gene. **A**, The most parsimonious tree (179 parsimony-informative characters, total length = 519, CI = 0.5934, RI = 0.8252 and, RC = 0.4897). **B**, Bayesian consensus tree. The support values: bootstrap values (MP) and posterior probabilities (BI) are shown on the branches. References to the sequences are given in Table 2.

Family ARIONIDAE Gray, 1840

The specimens had a mean of 10.07 mm of total length ($N = 3$; $SD = 0.74$; range = 9.5–10.9 mm). The external morphology and reproductive system of the dissected adult specimens were consistent with the Barker's descriptions (1999) for *Arion intermedius* Normand, 1852. Specimens from IFML 15449, 15566, 15568 (Table 1) were identified by external morphology. General coloration was golden yellow in most of the collected specimens, without lateral or central bands; ommatophores were dark brown; the mantle was oval with pneumostome on the right side in the antemedial portion; the posterior body section was rounded without keel. The slugs were collected under woodpiles or sheltering under rocks, in synanthropically disturbed locations, such as the service areas of Lago Puelo National Park, and the camping areas in Los Alerces National Park. The identification of the Arionidae specimen (MLP 13405) analyzed by molecular-genetic sequencing confirmed its specific identity as *A. intermedius*. Different phylogenetic analyses gave very similar topological organization for the NJ, MP, and ML trees and minor differences in the BI-tree organization (Fig. 3 cf. the MP and BI trees). The specimen from Argentina was placed

within the highly supported *A. intermedius* group (NJ = 100, MP = 100, ML = 100, BI = 1).

Family PHILOMYCIDAE Gray, 1847

Only the specimen from Puerto Iguazú was sexually mature (total length = 50 mm). The morphology of its reproductive system and external morphology coincided with descriptions by Tsai *et al.* (2005) and Gomes *et al.* (2011) for *Meghimatium pictum* (Stolyczka, 1873). Specimens from IFML 15570 A and MLP 13402 (Table 1) were identified by external morphology, which had an opaque beige background color of mantle, with two dark brown to black lateral stripes, and one medial stripe, often lighter than the lateral ones. This species was found in sites ranging from highly anthropically disturbed (in the Misiones and Tucumán provinces) to undisturbed areas (Iguazú National Park; Fig. 1, Table 1). The identification of Philomycidae specimens by DNA-data analysis confirmed their specific identity as *M. pictum* (MLP 13403 and USDA 110442; Table 1). The sequences obtained were identical and larger (655 bp) than previously reported for the species in South America (Gomes *et al.* 2011). All four phylogenetic approaches provided similar results and indicated a topology

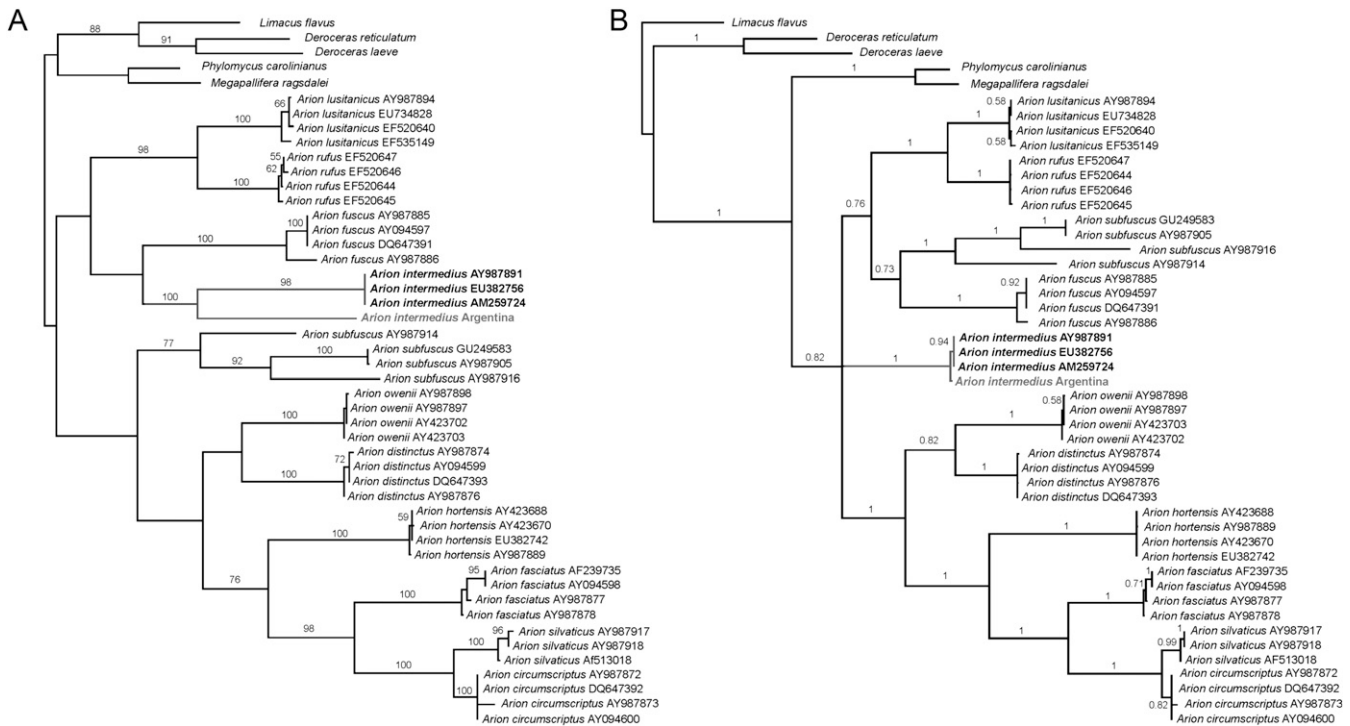


Figure 3. Phylogenetic trees of several invasive or potentially invasive *Arion* species in South America based on 552 nucleotides of the partial COI gene. **A**, One of the two most parsimonious trees (229 parsimony-informative characters, total length = 1074, CI = 0.4181, RI = 0.8085 and, RC = 0.3380). **B**, Bayesian consensus tree. The support values: bootstrap values (MP) and posterior probabilities (BI) are shown on the branches. References to the sequences are given in Table 3.

in agreement with Tsai *et al.* (2011; Fig. 4, cf. the MP and BI trees). In all instances, three groups were identified for *M. pictum*: Group 1 (NJ = 81, MP = 86, ML = 88, BI = 0.55) was geographically restricted to Taiwan Island (TWP1, TWP2, TWP3, TWP5, TWP6, TWP7, TWP8, TWP9 and TWP 10); Group 2 (NJ = 100, MP = 100, ML = 100, BI = 1) comprised *M. pictum* from Kinmen (TWP11) and Lieyu (TWP12) Islands as well as the South-American specimens (BRP1, BRP2, BRP3, BRP4, BRP5, ARP1 and ARP2) for which only a single haplotype was found. One sequence from southern Taiwan Island (TWP4) and a sequence from continental China (CNP2) were also found within Group 2. Finally, Group 3 (NJ = 99, MP = 91, ML = 100, BI = 0.81) included sequences from continental China (CNP1), Thailand (TLP1) and Matsu Island (*i.e.*, TWP13, TWP14, TWP15).

DISCUSSION

The present study revealed the existence of established populations of four exotic slugs in Argentina. The taxonomic identification of the alien species reported here is based on anatomical studies in addition to sequences of COI gene and

constitutes the first report of the presence of these species, all four of which have already shown an invasive behavior in several South American countries. *Deroceras panormitanum* (Lesson and Pollonera, 1882) and *Lehmannia valentiana*—both native of Europe—had been previously recorded for Chile and Colombia, while *L. valentiana* had also been reported in Brazil and Peru (Rumi *et al.* 2010). Based on anatomical and molecular evidence Reise *et al.* (2011) had split *D. panormitanum* into several species, including *D. panormitanum s.s.*, as well as *Deroceras invadens*, that latter as the single successful invader worldwide. According to this new taxonomic arrangement, the classification of *D. panormitanum* remains restricted to only the area where was originally described (*i.e.*, Malta and Sicily). *Arion intermedius*, a typical European Arionidae, has already been recorded in Chile and Colombia (Hausdorf 2002, Letellier *et al.* 2003, Cádiz and Gallardo 2007). *Meghimatium pictum*, a native from eastern and southern Asia, had recently been reported as an alien species in southern Brazil, with that record being the first one of the genus in South America (Gomes *et al.* 2011).

Lehmannia valentiana is the species with the oldest occurrence records in Argentina in comparison to the other three reported here, with data as early as 1924 (Buenos Aires

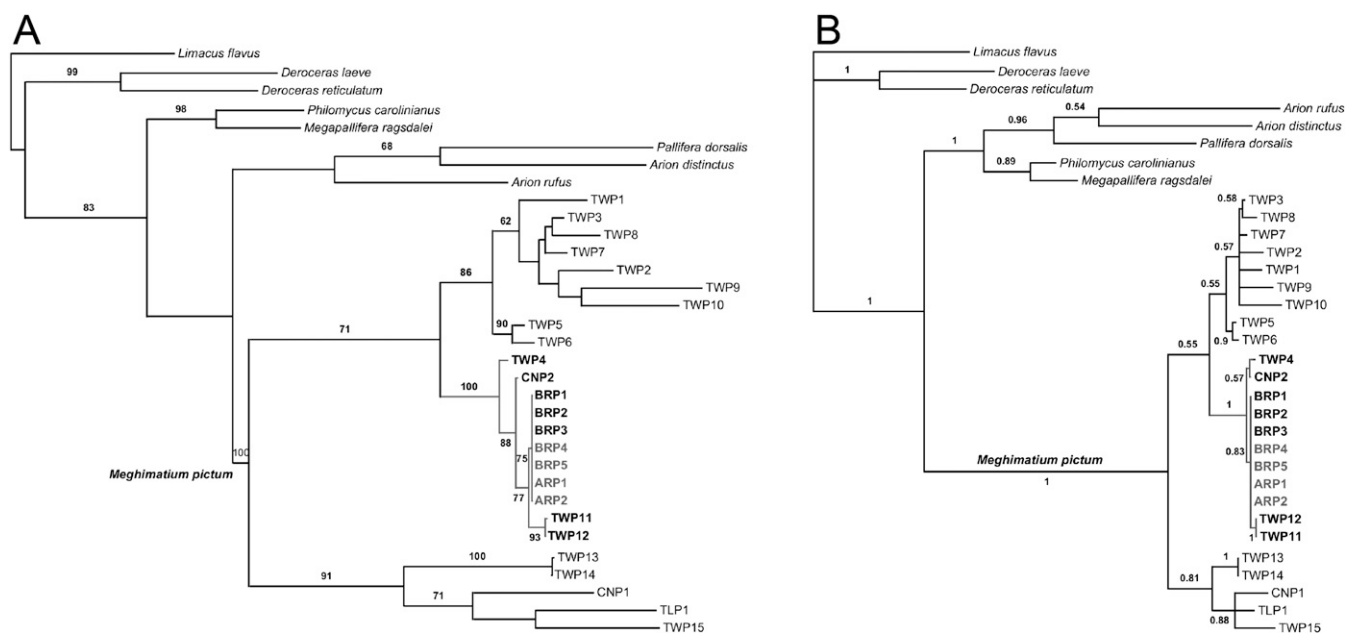


Figure 4. Phylogenetic reconstruction of *Meghimatium pictum*, based on 654 nucleotides of the partial COI gene. **A**, One of the six most parsimonious trees (245 parsimony-informative characters, total length = 987, CI = 0.464, RI = 0.6399 and, RC = 0.2969). **B**, Bayesian consensus tree. The support values: bootstrap values (MP) and posterior probabilities (BI) are shown on the branches. References to the sequences are given in Table 4.

province, MACN-In 14602) and 1962 (Tucumán province, IFML 604). To determine when and how this species was introduced into the country, however, is difficult because the molecular information at hand was not enough to shed any light on the area of origin of the species. *Lehmannia valentiana* could be considered as an established species, with an incipient invasive behavior since specimens have been recorded in remote sites (in both urban and protected areas), feeding on ornamental plants. In other countries, this species is considered to be a greenhouse pest, damaging orchid flowers and ornamental plants (Chichester and Getz 1969, South 1992, Ester *et al.* 2003).

Deroceras invadens was recorded in southern Argentina; but inferences about the site of the species' introduction were not possible because the sequence from Argentina grouped into a clade that included samples from diverse geographical origins (e.g., Canada, U.S.A., Germany, South Africa), thus indicating that the species had already been widely distributed worldwide. The specimens examined were from several towns of southern Argentina, a distribution that suggested the species had acquired an invasive behavior. The probable pathway for the introduction of *D. invadens* into Argentina could be related to commerce, as has been reported in the United States (Meissner *et al.* 2009). *Deroceras invadens* feeds on a great variety of plants and organic material in decomposition and is known to be a pest in pastures, gardens

and agricultural fields (Barker 1999). Moreover, this slug species is identified as an intermediate host for the nematode *Gallegostrongylus australis* Spratt, Haycock and Walter, 2001 (Spratt *et al.* 2001), which species parasitizes rodent lungs, causing mild pathologic changes.

We could neither clarify the source location nor assign a precise date of introduction for the specimens of *Arion intermedius* studied. Cádiz and Gallardo (2007) had reported this species for the first time in neighboring Chile, but the date cited could be considered controversial because Letelier *et al.* (2003) had recorded many specimens of *A. intermedius* deposited into National Museum of Natural History of Chile (but with no collection number) from southern Chile. We could nevertheless, conclude that populations of *A. intermedius* had been living in Argentina since at least 2003, the year when they were first collected (Table 1). This species can be considered to be established in Argentina since live specimens had also been found at the same locations seven years later, during the summer 2010 (Table 1). The pathway of introduction of the species into southern Argentina, however, remains unclear. Cádiz and Gallardo (2007) could not establish the introduction pathway into Chile, although they concluded that human accidental transport was the probable cause since species of that genus were being recovered from U.S. seaports on limestone, machinery and rocks (Meissner *et al.* 2009). *Arion intermedius* exhibits polyphagous or phytophagous

feeding habits and a predominant autofecundation, a strategy that increases its invasive capabilities (Wiktor *et al.* 2000, Reise *et al.* 2001). They are known to have a high capacity for penetrating undisturbed native forest, more so than any other type of exotic slug species (Hausdorf 2002, Cadiz and Gallardo 2007). In Argentina, occurrences of the species were recorded within national park areas, far away from urban environments. Within the Arionidae, the genus *Arion* Férussac, 1819 is recognized by international organizations concerned with pest control, such as the Eastern Region Cooperative Agricultural Pest Survey, as potentially damaging for agriculture (Cádiz and Gallardo 2007). Specimens could also act as intermediate hosts for nematodes such as *Filaroides martis* (Werner, 1783), a parasite that affects the respiratory tract of mammals (Grewal *et al.* 2003). In both the most parsimonious tree and the Bayesian consensus tree (Fig. 3), the *Arion* species analyzed grouped in a single clade, suggesting that phylogenetic analysis is as reliable a tool as the morphology in corroborating the identity of the different invasive members of the *Arion* genus.

The introduction of *Meghimatium pictum* into South America, and particularly into Brazil, was suggested to have been accidental through agricultural products in the 1990s, coinciding with the beginning of trade between China and Brazil in order to boost the production of mushrooms in the latter country (Gomes *et al.* 2011). On the basis of position of the South-American samples in all the topologies obtained in our phylogenetic reconstructions, we suggest that the origin of the South-American invasive lineage might be located in a region west of the Strait of Taiwan, either along the coast of China or within the islands close to that coast such as the Taiwanese Kinmen and Lieyu Islands. Currently, *M. pictum* can be found in several states of Brazil (Paraná, Santa Catarina and Rio Grande do Sul) bordering northeast Argentina and also in São Paulo State (Gomes *et al.* 2011). Occurrences of this species in the Misiones province of Argentina suggest that the introduction pathway into Argentina could possibly be linked either to an active dispersion of the slug from Brazil and/or to the commerce of flora between those aforementioned Brazilian areas and Argentina. That we recorded one specimen of *M. pictum* in a garden with orchids collected from the Misiones province plus other plants acquired from nurseries in the Tucumán province supports our hypothesis about the commercial trade as being the principal vector of introduction.

In Brazil, *Meghimatium pictum* was associated with the attack of plants in private gardens in Santa Catarina (Gomes *et al.* 2011) and has affected grape vines in Rio Grande do Sul (Baronio *et al.* 2011). In Argentina, further studies are required to know if incipient grape vines in Misiones province, covering about 50 hectares, are already affected; though, the extent of the invasion could be even worse if the species has

reached the western provinces (Mendoza, San Juan, La Rioja and Catamarca) where the 97% of the domestic production of wine is concentrated. Such a development could have serious implications on the country's national and international wine trade. In the Argentine areas where *M. pictum* is present, several other gastropods species (both natives and exotics) that can act as intermediate hosts for the zoonotic parasitic nematode *Angiostrongylus costaricensis* Moreira and Céspedes, 1971 can be found, with the potential risk that *M. pictum* could likewise begin to act as a new vector. *Meghimatium bilineatum* (Benson, 1842), a very close species to *M. pictum*, has furthermore been reported to be a host of *Angiostrongylus cantonensis* (Cheng, 1935) (Li *et al.* 2006), another zoonotic parasitic nematode.

We provide here the first report of four new exotic slugs in Argentine territories. Because of the invasive potential of these slug species, the present work provides new COI sequences obtained from morphologically confirmed specimens. We believe that the information contained in this report can help nonspecialists and government authorities quickly identify these species for the purpose of establishing guidelines for the prevention, control, and diffusion of those alien slugs.

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