Zootaxa 3691 (4): 453–460
www.mapress.com/zootaxa/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3691.4.4
http://zoobank.org/urn:lsid:zoobank.org:pub:E194B24D-140D-40E7-B5BD-89CC38E78A5D

The taxonomic status of *Deroceras hesperium* Pilsbry, 1944 (Gastropoda: Pulmonata: Agriolimacidae), a species of conservation concern in Oregon, USA

BARRY ROTH[1], ROBERT JADIN[2] & ROBERT GURALNICK[2]

[1]*745 Cole Street, San Francisco, California 94117, USA. E–mail: barry_roth@yahoo.com*
[2]*Dept. of Ecol. and Evol. Biol. and CU Museum of Natural Hist., E190A Museum Collections Bldg., University of Colorado, Boulder, Boulder, CO 80309–0265, USA. E–mail: rcjadin@gmail.com, Robert.Guralnick@colorado.edu*

Two native species of the slug genus *Deroceras* Rafinesque, 1820, have been identified in samples from Fremont–Winema National Forest and other national forests in the Pacific Northwest of the United States: (a) *Deroceras laeve* (Müller, 1774), common and widespread in North America (Pilsbry, 1948); and (b) *Deroceras hesperium* Pilsbry, 1944, thought to have a more restricted distribution and considered a species of special status by the US Forest Service and US Bureau of Land Management. Since at least 2004 the two species have been identified in previous samples on the basis of external appearance and features of the reproductive system. The localities for the two species are distributed in a mosaic, seemingly haphazard, pattern within the forests. Most samples previously examined and identified by author BR were assignable *in toto* to one or the other species, but both *D. hesperium* and *D. laeve* were identified in a sample from John Spring, Klamath County, Oregon. Specimens from central and southern Oregon counties represented an extension of the published range of *D. hesperium* southward from Oswego Lake, Clackamas County (Pilsbry, 1948; Branson, 1977).

The fact that two individuals differing in the diagnostic characters above were found at the same locality, along with the other distributional data, raises the question whether there are really two sympatric species or if the characters in question are variable within a population of a single species. In standard molluscan taxonomy, considerable weight is given to reproductive system differences, suggesting as they do that the bearers of different types of lower genitalia may be reproductively isolated from one another (e.g. Reise *et al.*, 2011).

This paper re-examines these two putative species, utilizing morphological and molecular evidence. Together these datasets should be competent to show whether slugs heretofore identified as a distinct species, *D. hesperium*, are in fact a separate, coherent, evolutionary lineage (a phylogenetic species), a discrete subset within the more inclusive species *D. laeve*, or merely individuals that happen to express an unusual, variant shape of their reproductive organs. We examined 164 specimens of *Deroceras* collected in six Oregon counties (Table 1; Figure 1). These were samples collected by Forest Service and Bureau of Land Management personnel in the course of their fieldwork. These specimens were deposited at the Oregon State Arthropod Collection (OSAC), Oregon State University, Corvallis, Oregon, and had been preserved in molecular grade ethanol.

Methods: Morphological Study. We inspected specimens from sampling localities designated for the study as D1 through D31 (Table 1). From one to five individuals from each locality were dissected. To avoid observer bias only one author, BR, performed all the morphological descriptions of the specimens and assigned the samples to one or the other species without knowing the molecular results. Characters that sort into alternate states (Table 2) were observed and recorded.

We excluded characteristics such as soft-tissue measurements because they are often unreliable taxonomically for reasons enumerated by Emberton (1989) and because they vary through an individual's ontogeny and perhaps in response to nutrition and time of year. *Deroceras laeve*, moreover, is genitally polymorphic, with some individuals never developing male terminal genitalia (Pilsbry, 1948; Jordaens *et al.*, 2006). We also excluded shape of the internal shell, particularly the curvature of the anterior margin, because it varies throughout the ontogeny of the individual, as can be seen from the growth lines preserved in the shell (compare Pilsbry, 1948:fig. 296F).

Accepted by J. Nekola: 2 Jul. 2013; published: 23 Jul. 2013

TABLE 1. Material studied, with locality information as supplied by OSAC.

Sample ID (CU)	OSAC Number (Barcode #) or Tracking #	Specimen Count	County (all: Oregon)	Locality Description	Coordinates, NAD27, Zone 10	
					UTM E	UTM N
D1	900000675	13	Klamath	Short Creek Rd. 3100	575684	4727713
D2	900000676	5	Klamath	Johnson Meadow #2	614201	4790811
D3	900000677	6	Klamath	Squirrel Camp 21	612112	4802195
D4	900000678	11	Lake	ED-3	698520	4704057
D5	900000679	11	Klamath	#18 Angel Springs	684937	4687772
D6	900000680	11	Klamath	Wilshire Spring/meadow	613825	4795052
D7	900000681	7	Lake	Leethomas CG Spring 3411 Rd.	677444	4717421
D8	900000682	9	Klamath	#7 off Sprague River Hwy	598867	4717872
D9	900000683	6	Lake	unit #8 Boulder Spring	683707	4727005
D10	900000684	9	Klamath	Rainbow Cr. Below DIM		
D11	900000685	10	Klamath	dry meadow enclosure	616555	4795881
D12	900000686	11	Klamath	#12 bottle spring	635404	4728445
D13	900000687	11	Klamath	#14 meadow and stream w. of 4400 Rd.	631887	4725404
D14	900000688	12	Klamath	upper Wilshire, north of 94 rd.	613570	4795497
D15	900000689	11	Klamath	Slabhouse Springs	603694	4731129
D16	WINII-19	1	Klamath	Buck	566255	4681474
D17	WINII-38	1	Lake	ED-3	698520	4704057
D18	900000660	1	Linn	[not supplied]	537824	4939522
D19	900000665	3	Lake	[not supplied]	740423	4658214
D20	900000654	1	Clackamas	[not supplied]	579129	5021948
D21	900000655	1	Lake	[not supplied]	729056	4697323
D22	900000662	1	Clackamas	[not supplied]	637659	4994776
D23	900000664	1	Linn	[not supplied]	541710	4941968
D24	900000661	1	Wasco	[not supplied]	621260	5029405
D25	900000666	3	Linn	[not supplied]	579994	4909631
D26	900000663	1	Jefferson	[not supplied]	n/a	n/a
D27	900000657	1	Lane	[not supplied]	595405	4916988
D28	900000658	1	Wasco	[not supplied]	624348	5013388
D29	900000653	1	Linn	[not supplied]	543702	4939454
D30	900000659	2	Clackamas	[not supplied]	559660	4978865
D31	900000656	1	Clackamas	[not supplied]	562762	4982153

Methods: Molecular Study. Genomic DNA was isolated from muscle tissue taken from 22 specimens of *Deroceras* using a Qiagen DNeasy extraction kit and protocol. Two mitochondrial gene fragments (cytochrome c oxidase subunit 1 (CO1) and 16S rRNA (16S) were independently PCR-amplified as described by Folmer *et al.* (1994) and Palumbi *et al.* (1991) using Promega GoTaq® Green master mix, the primer pairs: LCO1490 (F) + HCO2198 (R) and 16Sa (F) + 16Sb (R), respectively, with an annealing temperature of 48 ºC for both. Sequencing was performed in both forward and reverse directions using the PCR primers on a Beckman Coulter automated capillary sequencer, and sequence chromatographs were edited using Sequencher 4.2, Gene Codes Corporation, Ann Arbor, MI, USA. Sequences for each gene were aligned separately, first automatically using the program MUSCLE (Edgar, 2004), and then manually rechecked using Se-Al v2.0a11 (Rambaut, 2002). Gaps in alignments were treated as missing data. No internal stop codons were found in the protein-coding gene fragment CO1. Our new sequence data generated in this study (Figure 2, GenBank KF219885–918) were combined with data from six

species of *Deroceras* obtained from GenBank. For more distant outgroup taxa to root our *Deroceras* phylogeny, we downloaded from GenBank previously published sequences of the corresponding gene fragments of the marine basommatophoran species *Otina ovata* (Brown), found to be an adequate outgroup within the Pulmonata (Golding, 2012).

TABLE 2. Character states associated in the monograph of North American *Deroceras* by Pilsbry (1948) with *D. hesperium* and with *D. laeve*.

D. laeve	*D. hesperium*
Penis ovate, with medial swelling	Penis cylindrical, without medial swelling
Penis with medial constriction	Penis without medial constriction
Penis not narrowed above entry into atrium	Penis strongly narrowed just above entry into atrium
Penial gland (terminal appendage) recurved or spiral, but without mucronate (pointed) apex and without slender stalk	Penial gland recurved, with mucronate apex and separated from main body of penis by slender stalk
Spermathecal duct stout	Spermathecal duct slender
Mantle color (in EtOH) solid, blackish brown; or lighter brown, maculated over pale buff ground color	Mantle color (in EtOH) light brown, maculated over pale buff ground color
Dorsum color (in EtOH) solid, blackish brown to dark gray; or pale buff, more or less maculated with light brown	Dorsum color (in EtOH) pale buff, more or less maculated with light brown

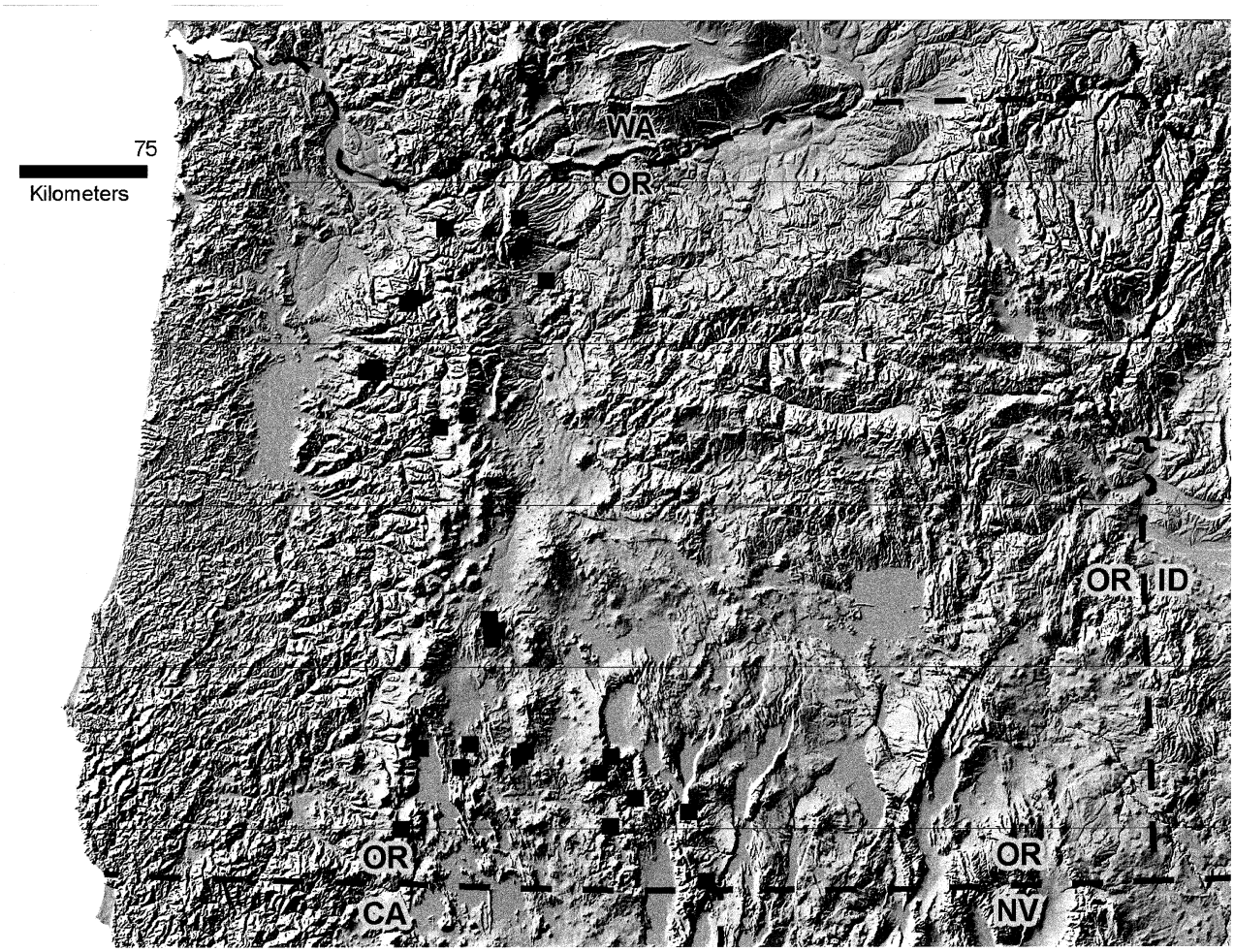

FIGURE 1. Relief map of Oregon showing the localities of *Deroceras* samples studied. CA=California, ID=Idaho, NV=Nevada, OR=Oregon, WA=Washington. Courtesy of Fremont–Winema National Forest.

Methods: Phylogenetic analysis. We used a mixed-model approach to reconstruct the phylogeny of *Deroceras* taxa with sequence data. To identify appropriate models of nucleotide substitution for both analyses, we used the program MrModeltest v2.2 (Nylander, 2004), run in PAUP* v4.0b10 (Swofford, 2002), which recovered the HKY + I model for the 16S gene and GTR + Γ, F81, and HKY + Γ models for 1st, 2nd, and 3rd, codon positions of CO1, respectively. We used Akaike information criterion (AIC) to select the best-fit models, as estimated by MrModeltest. The two gene fragments 16S (345 bp) and CO1 (630 bp) were concatenated and this combined dataset was partitioned by gene and codon position (for CO1), resulting in a total of four partitions.

We inferred phylogenetic relationships using Bayesian Inference (BI) criterion implementing MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003). Two simultaneous runs were conducted (with the default Markov chain Monte Carlo [MCMC] settings), for a total of 2.0×10^6 generations per run, sampling trees and parameters every 100 generations. We used potential scale reduction factor values (output by MrBayes), together with plots of cold chain likelihood values and parameter estimates visualized in Tracer v1.5.4 (Rambaut & Drummond, 2009) to confirm stationarity and convergence of MCMC runs. Based on this evaluation, the first 1.0×10^6 generations from each run were discarded as burn-in.

Using the same partitioning scheme described above, we inferred the ML tree using RAxML 7.2.8 and assessed tree support with the rapid-bootstrapping algorithm using 1000 non-parametric bootstrap values (Stamatakis, 2006; Stamatakis *et al.*, 2008). All ML estimates and tests were run under the GTRCAT model.

Results: Morphology. Table 3 shows the distribution of character states listed above and the species identification based on those characters. Some characters could not be scored in individual samples because the genitalia were not fully developed; this could be because a given specimen was immature, or because of genital polymorphism as mentioned above.

Where multiple specimens from a sample were dissected, all those with fully developed reproductive systems showed the genital character states indicated. The same consistency was shown for certain other characters. For instance, the two specimens from sample D9 both had large, globose spermathecas with slender spermathecal ducts. Three samples (D5, D8, and D19) had more than one external color pattern.

With some exceptions, the character states sort as predicted by the taxonomic literature. Those exceptions do show significant variability among samples and some specimens show mixed states (e.g. D5, D14, D16, D18). None of the specimens had a large, round swelling in the middle of the penis (such as shown for *D. laeve* by Pilsbry, 1948:fig. 291:1, 1a, 2); but those scored as *D. laeve* for penial shape had an ovate or fusiform, rather than parallel-sided, cylindrical penis as in *D. hesperium*. Pilsbry's own illustrations show considerable variation of this character in *D. laeve*. A uniformly brown pigmented dorsum and mantle have not been reported previously for *D. hesperium*, but sample D29 for which the internal characters uniformly score as HE has this external coloration. Sample D18 is exceptional in having a nearly equal mix of *D. hesperium* and *D. laeve* genital character states. A narrowed base of the penis, considered diagnostic of *D. hesperium* by Pilsbry (1948), occurs in several samples that otherwise have the penial features of *D. laeve*.

Results: Phylogenetic Analysis. Figure 2 shows the estimates of phylogenetic relationships among *Deroceras* species, indicating support for a within-species polytomy among Oregon *Deroceras* populations examined in this study. As is clearly visible, there are not two clear sets of deeply divergent clades with shallow within-clade tips that would support the concept of two different species as might be expected under a phylogenetic species concept.

Discussion and Conclusions: The benchmark for morphology-based taxonomy of North American species of the genus *Deroceras* is the monograph by Pilsbry (1948). In his treatment of the Holarctic species *D. laeve* (Pilsbry, 1948:539–552, figs. 289–293) he illustrated a wide range of genital morphologies from specimens across North America and some north Pacific islands. He synonymized numerous named species and forms with *D. laeve*, considering them to fall within the range of the species' variation. However, he retained *D. hesperium* as a separate species, based mainly on reproductive characteristics. On a strictly morphological basis, the results shown above don't fully contraindicate the existence of two morphotypes, one of them consistent with the diagnosis of *Deroceras hesperium*, the other falling within the range of the variable *D. laeve*. However, it is important to note that a few samples show a mixture of characters. Traditional taxonomy, perhaps implicitly weighting characters differentially, would tend to accept these results as indicating two species.

TABLE 3. Morphology-based identifications of samples. Character scores are those indicated in Materials and Methods, above; HE = state deemed typical for *D. hesperium*, LA = state deemed typical for *D. laeve*; HE/LA=state found in both *D. hesperium* and *D. laeve*; — = character not scored.

Sample ID (CU)	OSAC Number (Barcode #)	Tracking Number	Penis shape	Penial constriction	Penis narrowed	Penial gland mucronate	Spermathecal duct	Mantle color	Dorsum color	Identification
D1	900000675	n/a	HE	HE	HE	HE	HE	HE/ LA	HE/ LA	HE
D2	900000676	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D3	900000677	n/a	LA	LA	LA	LA	—	LA	LA	LA
D4	900000678	n/a	LA	LA	LA	LA	LA	HE/ LA	HE/ LA	LA
D5	900000679	n/a	LA	LA	HE	LA	LA	LA, HE/ LA	LA, HE/ LA	LA
D6	900000680	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D7	900000681	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D8	900000682	n/a	—	—	—	LA	—	HE/ LA	HE/ LA	LA
D9	900000683	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D10	900000684	n/a	HE	HE	HE	HE	HE	HE/ LA	HE/ LA	HE
D11	900000685	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D12	900000686	n/a	LA	LA	HE	LA	LA	LA	LA	LA
D13	900000687	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D14	900000688	n/a	HE	HE	LA	LA	LA	LA	LA	LA
D15	900000689	n/a	LA	LA	LA	LA	LA	LA	LA	LA
D16	n/a	WIN11-19	HE	HE	LA	—	HE	HE/ LA	HE/ LA	HE
D17	n/a	WIN11-38	LA	LA	HE	LA	LA	LA	LA	LA
D18	900000660	SAL06-017	HE	HE	HE	LA	LA	LA	HE/ LA	?
D19	900000665	FRE06-062	LA	LA	LA	LA	LA	HE/ LA	HE/ LA	LA
D20	900000654	SAL06-001	—	—	—	—	—	LA	LA	LA?
D21	900000655	FRE06-003j	HE	HE	HE	HE	—	HE/ LA	HE/ LA	HE
D22	900000662	SAL06-013	LA	LA	LA	LA	—	HE/ LA	HE/ LA	LA
D23	900000664	SAL06-011	HE	HE	LA	LA	HE	LA	LA	?
D24	900000661	MTH07-019	HE	LA	LA	LA	LA	LA	LA	LA
D25	900000666	WIL04-003	—	—	—	—	LA	HE/ LA	HE/ LA	LA
D26	900000663	DES01-002	HE	LA	LA	LA	LA	LA	LA	LA
D27	900000657	WIL06-009	—	—	—	—	—	HE/ LA	HE/ LA	?
D28	900000658	MTH07-006	Internal anatomy badly preserved					HE/ LA	HE/ LA	?
D29	900000653	SAL05-005	HE	HE	HE	HE	HE	LA	LA	HE
D30	900000659	SAL05-007	LA	LA	LA	LA	LA	LA	LA	LA
D31	900000656	SAL06-015	LA	LA	LA	LA	LA	LA	LA	LA

When mapped on the molecular tree (Figure 2), however, the three unquestioned *Deroceras hesperium* samples (D1, D10, and D29) do not form a clade but plot on separate branches. Unlike species-level divergences among other species sampled, which show clear genetic separation, there is no indication that *D. hesperium* and *D. laeve* form distinct clades. Sample D1 is more closely associated with *D. laeve* sample D7 than with any other *D. hesperium* sample and other samples morphologically identified as *D. hesperium* or *D. laeve* do not form strong inclusive clades to the exclusion of the other taxon. If the molecular tree is accepted as indicating phylogenetic relationships, then the hypothesis that the *D. hesperium* samples share a common ancestor not shared by any other

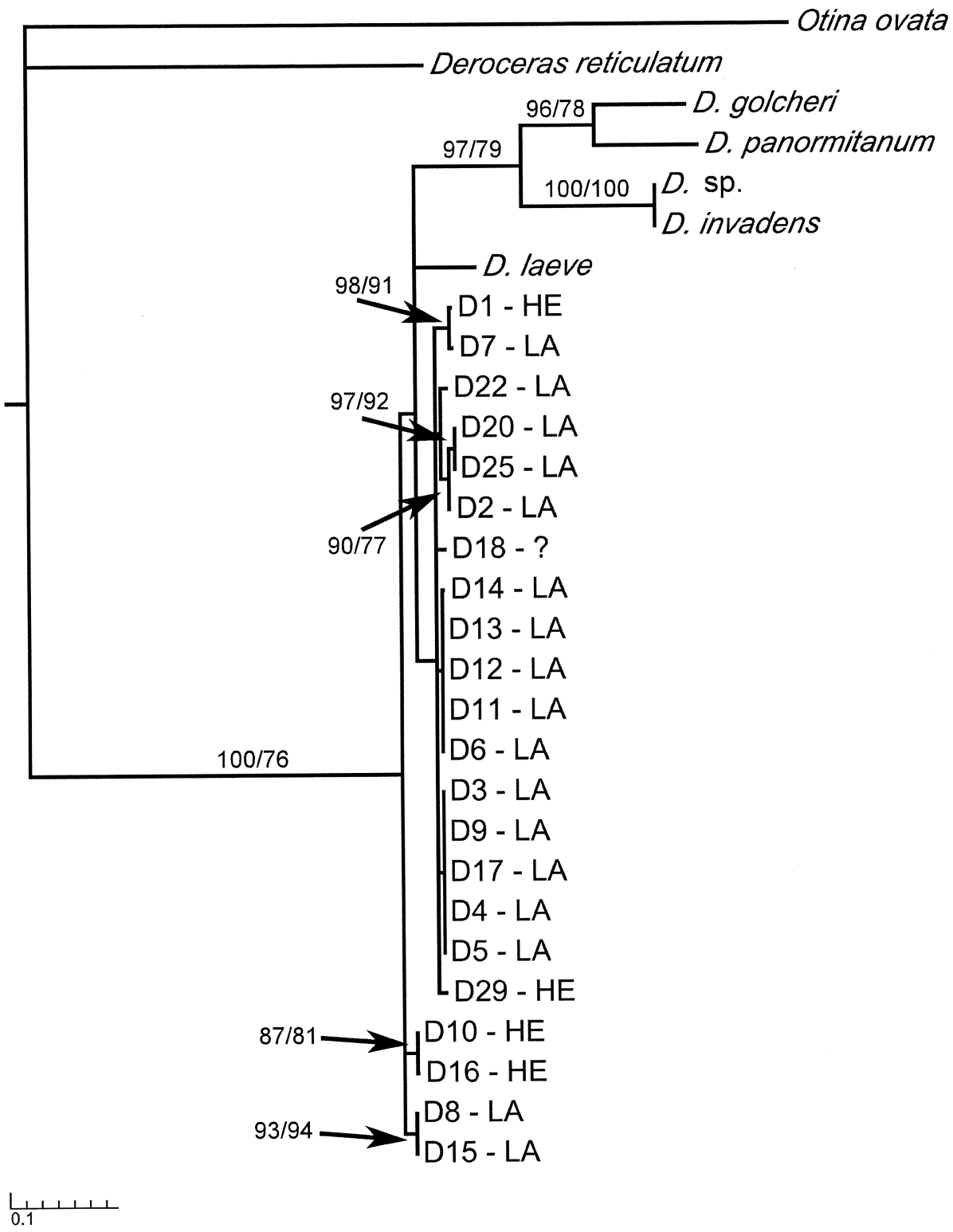

FIGURE 2. Bayesian 50% majority-rule consensus tree composed from a concatenated dataset (CO1 and 16S; total of 975 bp) showing relations among *Deroceras* species, including samples morphologically identified as *D. laeve* (LA), *D. hesperium* (HE) or ambiguous (?). Numbers at nodes represent values of Bayesian posterior probabilities (PP, left) and Maximum Likelihood Bootstrap values (BS, right). Nodal support values ≥ 95% PP and ≥ 70 BS are illustrated and considered highly supported. Taxa and GenBank accession numbers: *Deroceras golcheri* Altena, CO1 JN248292; *D. invadens* Reise, Hutchinson, Schunack & Schlitt, CO1 JN248314; *D. laeve*, CO1 HM584699; *D. panormitanum* (Lessona & Pollonera), CO1 JN248311; *D. reticulatum* (Müller), CO1 FJ917286, 16S FJ917266; *D.* sp.[1], CO1 FJ358222; *Otina ovata* (Brown) (Otinidae), CO1 F489389, 16S EF489310.

samples in the analysis is falsified. Under at least one widely accepted phylogenetic definition of a species,[2] therefore, the *D. hesperium* samples in this analysis would not qualify as a species distinct from *D. laeve*. An alternative interpretation is that the morphotype here designated *D. hesperium* represents a largely covarying set of character states that occurs sporadically within the range of the widespread *Deroceras* species in Oregon, i.e., *D. laeve*.

The single *D. laeve* sample, from Kentucky, USA, for which there was an existing GenBank accession for the cytochrome oxidase I gene, while having a partly unique set of apomorphies, is found within a clade that includes our *D. laeve* samples. However this clade also includes other *Deroceras* species and we cannot establish monophyly of the widespread *D. laeve* based on this analysis alone.

This study did not examine nuclear genes, and it remains possible that mitochondrial introgression has occurred and nuclear genes may show a different phylogenetic pattern. Further work utilizing nuclear markers is therefore warranted in order to verify the results reported here. The preponderance of other evidence is best interpreted as indicating a variable, single species. Thus, *Deroceras hesperium* should be considered a junior synonym of *Deroceras laeve*.

Acknowledgments

This work was carried out under USDA Forest Service Purchase Orders AG-04P5-P-11-0008 and 11-CR-11060200-013. The molecular component was performed by RCJ and RPG and the morphological component by BR. A synthesis of results was produced by all investigators working together. We thank Sarah M. Malaby for overseeing the project development; Darci Rivers-Pankratz, Missy Anderson, Amy Markus, Kelli J. Van Norman, DeEtte M. Stofleth, and Deanna Vest for invaluable organizational assistance; Heather Robeson for assistance with molecular work in the laboratory; and Gene Hall for specimen management. The submitted manuscript was improved by reviews from T. Backeljau and one anonymous reviewer.

References

Branson, B.A. (1977) Freshwater and terrestrial Mollusca of the Olympic Peninsula, Washington. *The Veliger*, 19, 310–330.

de Queiroz, K. (2005) Different species problems and their resolution. *BioEssays*, 27, 1263–1269. http://dx.doi.org/10.1002/bies.20325

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797. http://dx.doi.org/10.1093/nar/gkh340

Emberton, K.C. (1989) Retraction/extension and measurement error in a land snail: Effects of systematic characters. *Malacologia*, 31, 157–174.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Golding, R.E. (2012) Molecular phylogenetic analysis of mudflat snails (Gastropoda: Euthyneura: Amphiboloidea) supports an Australasian centre of origin. *Molecular Phylogenetics and Evolution*, 63, 72–81. http://dx.doi.org/10.1016/j.ympev.2011.12.016

Jordaens, K., Pinceel, J. & Backeljau, T. (2006) Life-history variation in selfing multilocus genotypes of the land slug *Deroceras laeve* (Pulmonata: Agriolimacidae). *Journal of Molluscan Studies*, 72, 229–233.

Nylander, J.A.A. (2004) *MrModeltest v2.2*. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.

1. Reise *et al.* (2011: fig. 9) reported this unidentified *Deroceras* species as “tramp species” and later (:220) described the “tramp species” as *D. invadens*.
2. A separately evolving metapopulation lineage, where a metapopulation is an inclusive population made up of a set of connected subpopulations and a lineage is a population extended through time or an ancestral-descendant series of time-limited (instantaneous) populations (de Queiroz, 2005:1263).

Palumbi, S.R., Martin, A.P. , Romano, S.L., McMillan, W.O., Stice, L. & Grabowski, G. (1991) *The Simple Fool's Guide to PCR. Department of Zoology*, University of Hawaii, Honolulu.
Pilsbry, H.A. (1944) West American field slugs (*Deroceras*). *The Nautilus*, 58, 15–16.
Pilsbry, H.A. (1948) Land Mollusca of North America (north of Mexico). *Academy of Natural Sciences of Philadelphia Monograph*, 3, 2(2), i–xlviii, 521–1113.
Rambaut A. (2002) *Se-Al: Sequence alignment editor, Version 2.0a11*. Available at http://tree.bio.ed.ac.uk/software/seal/
Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5.* Available at http://tree.bio.ed.ac.uk/software/tracer/ (Accessed 1 July 2013)
Reise, H., Hutchinson, J.M., Scunack, S. & Schlitt, B. (2011) *Deroceras panormitanum* and congeners from Malta and Sicily, with a redescription of the widespread pest slug as *Deroceras invadens* n. sp. *Folia Malacologica*, 19, 201–223.
http://dx.doi.org/10.2478/v10125-011-0028-1
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
http://dx.doi.org/10.1093/bioinformatics/btg180
Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
http://dx.doi.org/10.1093/bioinformatics/btl446
Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology*, 75, 758–771.
http://dx.doi.org/10.1080/10635150802429642
Swofford, D.L. (2002) *PAUP*: Phylogenetic analyses using parsimony (*and other methods)*, version 4.0b10. Sunderland, MA: Sinauer Associates.