Introduced *Prophysaon andersonii* (J.G. Cooper, 1872) in Quebec, Canada: first record of *Prophysaon* (Gastropoda, Eupulmonata, Arionoidea) in eastern North America, confirmed by partial-COI gene sequence

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**Abstract**

We report for the first time the terrestrial slug *Prophysaon andersonii* (J.G. Cooper, 1872) from Quebec, Canada. Two specimens were collected in Parc national du Bic. The identification was determined by the external morphology and partial-COI gene sequence data. The genus *Prophysaon* is endemic to western North America, and the new record indisputably represents an introduction. No species of *Prophysaon* has, until now, been noticed in North America from outside its native range.

**Keywords**

Cytochrome c oxidase subunit I, introduced species, Mollusca, Reticulate Taildropper, terrestrial slugs.

**Introduction**

The genus *Prophysaon* Bland & W.G. Binney, 1873 is endemic to western North America, distributed from Attu Island, Aleutian Islands, Alaska (Roth and Lindberg 1981) to California and east to Idaho and Montana (Pilsbry 1948; Smith et al. 2018). This genus has been traditionally placed either in a broadly defined family Arionidae (e.g. Pilsbry 1948) or in the family Anadenidae, when it is raised from its traditional subfamilial rank within the Arionidae (e.g. Wiktor 2000; Bouchet et al. 2017). Pilsbry (1948) divided the genus into two subgenera and recognized eight species. These slugs are known as “taildroppers” on account of their ability to autotomize the end of their tail as a defensive behaviour (Hand and Ingram 1950; Stasek 1967; Deyrup-Olsen et al. 1986). In Canada one species, *P. coeruleum* Cockerell, 1890, was assessed by COSEWIC (2016) as Endangered; the other three Canadian species—*P. andersonii* (J.G. Cooper, 1872), *P. foliolatum* (Gould in A. Binney, 1851), and *P. vanattae* Pilsbry, 1948—are apparently not at risk.

Recently, Proschwitz et al. (2017) documented *P. foliolatum* (Gould in A. Binney, 1851) in southern Sweden, introduced from North America with the trade of salal (*Gaultheria shallon* Pursh) for the floral industry. One of
us (RGF) has observed *P. andersonii* in association with strongly synanthropic, mostly European, introduced terrestrial slugs and snails in retail plant nurseries in British Columbia. This suggests that at least some members of the genus are likely candidates for introduction elsewhere by the movement of nursery stock and soil. Although the introduced status of at least one *Prophysaon* species has now been confirmed in Europe (southern Sweden; Proschwitz et al. 2017), there have surprisingly not been any reports of this genus introduced to anywhere in Canada or the eastern USA. Nevertheless, the species was found in southern California, 630 km south of its previously known southernmost limit in central California (Pearce and Richart 2010).

We use partial-COI gene sequence data to complement morphological species identification and report *P. andersonii* from Quebec. This appears to be the first record of any *Prophysaon* species from eastern North America.

**Methods**

The present paper is a result of a general reconnaissance of several Quebec national parks in Sept. 2019 by one of us (AN). The geographic position of the collection site of the new record of *Prophysaon andersonii* was obtained using a GPS receiver (Garmin eTrex 30x, Southampton, UK). Specimens were collected by hand and placed directly in 95% ethanol for barcoding and deposited at the Biodiversity Institute of Ontario (BIOUG), Guelph, Ontario, Canada. These specimens were assigned to the project with the project code FTMC in the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org, Ratnasingham and Hebert 2007).

To map the species’ distribution, GBIF (2019) was searched for occurrence data and the results included 535 usable records, from 20 published datasets (https://doi.org/10.15468/dl.dhkjfe). Added to these were 73 unpublished records held by one of us (RGF, Appendix), as well as one record from Pearce and Richart (2010), 81 records from Smith et al. (2018), and the new record.

For the phylogenetic analysis, *Anguispira alternata* (Say, 1817) (Discidae) (N = 13) from the FTMC project was selected as the outgroup. Samples of foot tissue from *P. andersonii* and *A. alternata* were used for molecular analysis following protocols in Layton et al. (2019). In this protocol the barcode region of cytochrome c oxidase subunit I (COI) was amplified with the universal “Folmer” primers (LCO1490/HCO2198) (Folmer et al. 1994) and sequenced. After editing and aligning the sequences their quality was checked. The sequence dataset for the genus *Prophysaon* was completed by additional sequence data from GenBank and from various projects on BOLD. The Barcode Index Number (BIN) algorithm was applied to delineate clusters corresponding to operational taxonomic units at the species level (Ratnasingham and Hebert 2013).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. This analysis involved 341 nucleotide sequences (of which 13 were *Anguispira alternata* serving as outgroup). Sequences were mined from Genbank and BOLD. In Genbank, the majority of sequences came from western North America (Smith et al. 2018) and one sequence came from Sweden, a specimen of the introduced *Prophysaon foliatum* (Proschwitz et al. 2017). All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 655 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

**Results**

*Prophysaon andersonii* (J.G. Cooper, 1872)

*Arion? Andersonii* J.G. Cooper 1872: 148, pl. 3, figs 1–5. Since at least Pilsbry (1948), this species is frequently misspelled *andersoni*. However, according to the Code (ICZN 1999), the original spelling *Andersonii* should be emended to *andersonii* (Article 32.5.2.5), but the -ii termination must not be changed to a single i (Article 33.4).

**New record** (Fig. 1). CANADA: Quebec • 2 specimens; Bas-Saint-Laurent: municipalité régionale de comté du Rimouski-Neigette: Parc national du Bic: Anse à Wilson; 48.3597°N, 068.7983°W; 10 m elev.; 9 Sept. 2016; A. Nicolai leg.; BIOUG37881-C05 (FTMCA774-18).

**Identification** (Fig. 2). The external appearance of the slugs conforms to the characters of the genus *Prophysaon*. Mantle anterior (shell not visible externally) and coarsely granular, with pneumostome in front half of mantle. Tail rounded, not keeled, without mucus gland at tip, and with line of abscission of autotomized tail faintly visible. Sides of body strongly reticulated. In alcohol, body brownish, with darker reticulations, and with one dark lateral band on each side of mantle. Pale dorsal stripe running down length of tail. Length of largest slug (Fig. 2; contracted, in ethanol): 22 mm.

*Prophysaon andersonii* and *P. foliatum* are similar in appearance. Colour of the skin in these species varies considerably, especially in *P. foliatum* which typically has the mantle rimmed with bright yellow (Burke 2013). However, the pigmentation of our Quebec specimens, now that they have been in alcohol since their collection, cannot be relied on. No photographs of or notes on the Quebec specimens while alive were taken, but the slugs were immediately recognized as *P. andersonii* at the time. The preserved slugs, now discoloured, faded, and strongly contracted in alcohol do not readily show the main characters used in distinguishing these species when alive. *Prophysaon andersonii* is decidedly smaller than *P. foliatum*, with the length of 30–60 mm when adult and in a normal, extended position (Burke 2013); *P. foliatum* is 50–80 mm (Burke 2013) to over 100 mm (Pilsbry 1948). Assuming the Quebec specimens are
fully grown, or nearly so, in their uncontracted state, they would have been within the size range expected for *P. andersonii*, or at the smallest end of the range for *P. foliolatum*. The autotomized portion of the tail is somewhat longer and the line of abscission more distinctly marked in *P. foliolatum* than in *P. andersonii* (Pilsbry 1948; Burke 2013). In our specimens, the line of abscission is hardly visible (Fig. 2), but perhaps a little more like *P. andersonii* than *P. foliolatum* in its position on the body, as compared with the illustrations of preserved (contracted) slugs of these species published by Pilsbry (1948: figs 372, 375). The reticulated pattern on the tail is also somewhat less coarse in *P. andersonii* than in *P. foliolatum*. Although it is difficult to tell for certain, our specimens look to have the finer reticulation of *P. andersonii*—again, in comparison with Pilsbry’s (1948) figures 372 and 375.

Pilsbry (1948) described and illustrated the anatomy of the reproductive system of both species, although the number of specimens studied does not seem to have been great. In the nominotypical subgenus the epiphallus is abruptly enlarged into an oblong, muscular body; Pilsbry (1948) described this structure in *P. andersonii* as more or less straight, whereas in *P. foliolatum*, it was found to be slightly curved. He also observed that the slender
part of the epiphallus in *P. foliolatum* is extremely long, apparently longer than in *P. andersonii*. Although Roth and Lindberg (1981) and Forsyth (2001) have since mentioned for other specimens the enlarged, muscular portion of the epiphallus, there has been no comprehensive investigation to evaluate the usefulness of these observed differences in determining species. We did not attempt the dissection of our material because we felt this would not tell us anything. It is of note that Pilsbry (1948) did not use any anatomical characters in distinguishing *P. andersonii* and *P. foliolatum* in his key; perhaps he did not place much importance on the data he had.

The BIN AAU1161 was assigned to the COI sequence of the *Prophysaon* specimen from Quebec which falls within the *P. andersonii* cluster where some other specimens from British Columbia have the same BIN (Fig. 3). Although the COI genes seem to be highly variable in this genus (Smith et al. 2018), introduced *P. andersonii* in Quebec and *P. foliolatum* in Sweden (Proschwitz et al. 2017) can be clearly associated with the clusters of specimens from North America of these species. The node for the *P. andersonii* cluster has a support value of 65%, while the node of the *P. foliolatum* cluster has a support value of 98%.

Discussion

In Canada, *Prophysaon andersonii* is native to British Columbia, where it occurs along both coastal and interior areas (Forsyth 2004, 2005; Fig. 1). Among the species of *Prophysaon*, this one has the broadest geographic range, from Alaska to California and east to Idaho and Montana (Pilsbry 1948; Roth and Lindberg 1981; Smith et al. 2018), and thus, perhaps the greatest adaptability. In British Columbia, *P. andersonii* lives in forests mostly, but has been seen in somewhat disturbed habitats (Forsyth 2004), as well as in retail plant nurseries, where it co-occurs with introduced and anthropophilic terrestrial snails and slugs (Forsyth pers. obs.). While the origin and means of transport of *P. andersonii* to Quebec are unknown, it seems probable to us that this species made its way there via nursery stock.

Parc national du Bic is located on the south shore of the St Lawrence River estuary, ca 15 km south-west of the city of Rimouski. The park has forested, rocky promontories connected by lowlands, which are either forested as well or have old fields. Within the park there are well-developed recreation facilities, including trails and campgrounds. Therefore, the new record at that park could have been introduced as campers. For example, the introduced slugs *Deroceras reticulatum* (O.F. Müller, 1774) and several species of the genus *Arion* A. Férussac, 1819 are common in British Columbia campsites, including some in quite remote areas (Forsyth pers. obs.), and these seem likely to have been transported there by movement of tents (Grimm et al. 2010) and also possibly firewood. It is possible that *P. andersonii* has been transported to Parc national du Bic in that way, although we are not suggesting that cross-country transport was by this means. Another possible means of transport of either slugs or their eggs is by flowing water.

Wherever the local source of *P. andersonii* to Parc national du Bic might have been, it seems likely that it was nearby. We suspect that this species might occur elsewhere in the area, and perhaps *P. andersonii* is more commonly introduced in eastern Canada than currently realised. Investigations of parks, gardens, and plant nurseries in Quebec, and more generally in eastern Canada and the north-eastern USA, might locate additional records of this species.

Acknowledgements

We thank the Centre for Biodiversity Genomics in Guelph (Ontario) for their kind support and partially funding of this study, and especially Kara Layton, Angela Telfer, Jayne Sones, Jeremy deWaard, and Paul Hebert who helped with genetic analyses and/or generally supported our efforts of terrestrial gastropod barcoding in Canada. We especially thank Timothy Pearce, Barry Roth, and John Hutchinson, as well as the editor, Bárbara Romera, for their comments and suggestions for improvements to the manuscript. We thank Donald McAlpine for reviewing our manuscript after acceptance and the New Brunswick Museum (Saint John, New Brunswick) for its contribution towards a portion of the article processing charge.

Authors’ Contributions

AN collected the specimens, analysed the COI gene sequences, and prepared Figure 3. RGF photographed the specimen and prepared Figures 1 and 2 and the Appendix. AN and RGF shared in writing and revision of the text.

References


Cooper JG (1872) On new Californian Pulmonata, etc. Proceedings of the Academy of Natural Sciences of Philadelphia 24 (2): 143–154, pl. 3.

Figure 3. Evolutionary relationship of Prophysaon species. Indicated at each branch are from left to right: record code | species name as identified | Barcode of Life Data Systems sample ID | country | province or state | Barcode Index Number | Genbank accession number. The single shortest branch length = 3.1982518 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. (Figure continued on next pages.)
| GBMLG0795-06 | | United States | Idaho | BOLD:AAX7849 | AY357614 |
| GBMLG0794-06 | | United States | Washington | | United States | Idaho | BOLD:AAA9104 | AY357620 |
| GBMLG0799-06 | | United States | Oregon | BOLD:ACF3878 | AY357638 |
| GBMLG0809-06 | | United States | Washington | | United States | Washington | BOLD:ACE4323 | AY357624 |
| GBMLG0818-06 | | United States | Oregon | BOLD:AAU1161 | NEW RECORD FROM QUEBEC |
| GBMLG0801-06 | | Canada | Quebec | BOLD:AAU1161 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 49.50 | 27.49 | 2171 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 49.50 | 27.49 | 003 00161 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| 49.50 | 27.49 | 3338 | | United States | Idaho | BOLD:AAU1161 | KM612009 |
| 49.50 | 27.49 | 3251 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| 49.50 | 27.49 | 2204 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 2179 | | United States | Washington | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 3270 | | United States | Washington | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 3338 | | United States | Idaho | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 3251 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 2204 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 2171 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 55.60 | 30.59 | 003 00161 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 3270 | | United States | Washington | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 3338 | | United States | Idaho | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 3251 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 2204 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 2171 | | United States | Oregon | BOLD:AAU1161 | KM612009 |
| 62.69 | 38.68 | 003 00161 | | Canada | British Columbia | BOLD:AAU1161 | KM612009 |

**Figure 3. Continued.**
Figure 3. Continued.


Appendix

Unpublished records of Prophysaon andersonii collected, recorded, or held by R. Forsyth. These supplement the GBIF data used to produce Figure 1. Abbreviations: RGF = field number or collection of R. Forsyth; RBCM = Royal British Columbia Museum (Victoria, British Columbia, Canada).

CANADA, British Columbia • observation; Fraser Valley, Mission, Fraser River, NW of mouth of Silverdale Creek; 49.14, –122.3803; 9 June 2001; R. Forsyth, T. Forsyth obs.; in low cottonwood grove adjacent to river; RGF 01.004 • observation; Alberni-Clayoquot Regional District, Port Alberni, Paper Mill Dam Park; 49.2735, –125.8551; 5 June 2015; R. Forsyth leg.; along edge of Western Redcedar forest, under dead wood; RGF 15.041 • spec.; Bulkley-Nechako Regional District, Hazelton Mountains, Bulkley Range, Goat Creek, ca 0.5 km SE of Four Creek; 54.6173, –127.1328; 699 m elev.; 16 Aug. 2004; R. Forsyth, T. Forsyth leg.; open, disturbed roadside; under timbers and boards of old bridge; RGF 04.023, RBCM 004-00148-002 • observation; Bulkley-Nechako Regional District, Hazelton Mountains, Bulkley Ranges, Telkwa Pass, E end of Top Lake, near small creek flowing in from N; 54.5743, –127.7005; 850 m elev.; 25 Aug. 2001; R. Forsyth leg.; on pieces of fallen bark, low wet ground along shore; RGF 01.017 • observation; Vancouver Island, Capital Regional District, Saanich Peninsula, Central Saanich, Island View Park, Island View Road at Homathko Road; 48.5727, –123.366; 12 Oct. 2001; R. Forsyth, H. Reise, J. Hutchinson leg.; litter, under crabapple and wild roses; RGF 01.030 • observation; Vancouver Island, Capital Regional District, Saanich, Prospect Park, Estelline Road; 48.5112, –123.468; 50 m elev.; 12 Oct. 2001; H. Reise, J. Hutchinson, R. Forsyth leg.; wet, deciduous woods, with Skunk cabbage, on sticks, leaf litter; RGF 01.033 • observation; Vancouver Island, Capital Regional District, San Juan River valley, near Fairy Lake recreation site, NE of Port Renfrew; 48.5878, –123.352; 11 Oct. 2001; H. Reise, J. Hutchinson, R. Forsyth leg.; on fallen Bigleaf Maple leaves; alder-hemlock forest; RGF 01.039 • observation; Vancouver Island, Comox-Strathcona Regional District, Union Bay, Greene Avenue; 49.5713, –124.897; 100 m elev.; 11 Nov. 2001; R. Forsyth, T. Forsyth leg.; second-growth Douglas-fir forest, Red Alders along road, Thimbleberry, Salmon-berry, Bigleaf Maple; RGF 01.048 • observation; Vancouver Island, Cowichan Valley Regional District, North Cowichan, Eves Park; 48.86, –123.695; 10 Nov. 2001; R. Forsyth, T. Forsyth leg.; old-growth coniferous forest along creek, under stone; RGF 01.042 • observation; Skeena-Queen Charlotte Regional District, Haida Gwaii, Graham Island, Naikoon Provincial Park, near Tow Hill; 17 May 2000; T. Forsyth leg.; edge of campsite, under plywood on grass and moss; surrounded by pine and Salal; RGF 00.053 • spec.; Columbia-Shuswap Regional District, Rocky Mountain Trench, Hwy 95, Braisher Rest Area; 51.1117, –116.7125; 862 m elev.; 19 Sept. 2008; R. Forsyth leg.; RGF 08.149 • 1 spec.; Fraser-Fort George Regional District, Nadsilich (West) Lake, West Lake Provincial Park, near boat launch; 53.7332, –122.8573; 23 June 2001; P. Lambert, R. Forsyth leg.; Trembling Aspen alone lake, many juveniles; L01-45, RBCM 001-00333-001 • 1 spec.; Shahtka Lake, Paarans Beach Provincial Park; 54.4206, –124.3783; 22 June 2001; P. Lambert, R. Forsyth leg.; dense shrubs
with deep, moist litter, Trembling Aspen; L01-36, RBCM 001-00037-001 • 1 spec.; East Kootenay Regional District, W of Galloway, Hwy 3/93, near Sand Creek, Betania Road; 49.3705, −115.2432; 19 Aug. 2002; R. Forsyth et al. leg.; under litter; RGF 02.033, RBCM 002-000163-001 • 1 spec.; Vancouver Island, Cowichan Valley Regional District, Bamberton Provincial Park; 13 Oct. 2001; H. Reise, J. Hutchinson, R. Forsyth, T. Forsyth leg.; Douglas-fir, maple, Braham Rest Area, near Johnson Draw Creek; 51.1115, −116.7114; 811 m elev.; 22 Aug. 2018; A. Nicolai, R. Forsyth leg.; Douglas-fir, Bigleaf Maple woods, on surface of ground; RGF 01.037.4899, RBCM 002-000120-001 • 1 spec.; East Kootenay Regional District, Rocky Mountain Trench, E of Fort Steele, flats, right bank, Wild Horse Creek below Wardner-Fort Steele Road; 49.6188, −115.6213; 802 m elev.; 13 Aug. 2012; R. Forsyth leg.; under pieces of wood; cobby, rather open site with young cottonwoods and pine; RGF 12.087.6212 • 1 spec.; Fraser Valley Regional District, Cascade Mountains, Skagit Range, Chilliwack Lake, near mouth of Depot Creek; 49.0267, −121.4; 610 m elev.; 30 Apr. 2000; R. Forsyth, K. Ovaska, L. Sopuck leg.; low ground; large cottonwoods, under bark and dead sticks; RGF 00.027.4844, RBCM 000-000137-001 • 1 spec.; North Okanagan Regional District, Interior Plateau, Okanagan Highland, Vernon-Slocan Hwy 6 W of Cherryville; 50.2731, −118.793; 522 m elev.; 12 Aug. 2012; R. Forsyth leg.; Douglas-fir with Douglas Maple forest, under bark of cut log in woods; RGF 12.081.6055 • 1 spec.; Skeena-Queen Charlotte Regional District, Haida Gwaii, Graham Island, Yakoun Lake; 53.341, −132.421; 27 Apr. 2000; K. Ovaska leg.; old growth coniferous forest, Sitka Spruce, hemlock, within litter; RGF 00.033.4776 • 1 spec.; Skeena-Queen Charlotte Regional District, Haida Gwaii, Graham Island, Rennell Sound, Shelly Creek; 53.3467, −132.4267; 23 May 2000; T. Forsyth leg.; under logs, other wood, and rocks, mostly alder; RGF 00.058.4868, RBCM 003-00038-002 • 1 spec.; Thompson-Nicola Regional District, Charcoal Creek at Chase-Falkland Road; 50.6362, −119.6573; 830 m elev.; 21 July 2013; J. Hutchinson, H. Reise, R. Forsyth leg.; near creek; mixed wood riparian; RGF 13.065.6629 • 2 spec.; Kitimat-Stikine Regional District, Observator Inlet, Alice Arm, Kitsault, W of Lime Creek; 55.456, −129.4855; 23 June 2002; R. Forsyth, T. Forsyth leg.; open, disturbed site; campsite, and under driftwood along shore; RGF 02.015, RBCM 002-000146-004 • 3 spec.; Fraser-Fort George Regional District, Rocky Mountain Trench, Mackenzie, Morfee Lakes, South Lake; 55.3404, −123.075; 740 m elev.; 18 Aug. 2003; R. Forsyth leg.; NE slope with young alder <1 m high; RGF 03.086, RBCM 003-000161-001 • 3 spec.; Cariboo Regional District, W of Quesnel, Nakko Highway, vicinity of Baker Creek; 52.9753, −122.8075; 20 June 2001; P. Lambert, R. Forsyth leg.; roadside; Trembling Aspen and pine, grassy, under wood, stumps, and logs; RGF L01-17, RBCM 001-00032-001 • 3 spec.; Skeena-Queen Charlotte Regional District, Haida Gwaii, Moresby Island, Moresby Camp; 53.051, −132.03; 19 May 2000; T. Forsyth leg.; disturbed site; alder, spruce nearby; under boards on grass; RGF 00.056 • 4 spec.; Vancouver Island, Cowichan Valley Regional District, near unnamed creek, S of Bamberton Park; 48.6038, −123.5207; 1 m elev.; 15 May 1999; R. Forsyth, T. Forsyth leg.; under logs, planks and small pieces of wood, adjacent to beach; RGF 99.033.4630, RBCM 001-000323-001.