

## Not in the least concern: anthropogenic influences on a South-east Asian apple snail *Pila scutata* (Ampullariidae)

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SUPPLEMENTARY TABLE 1 Historical localities of *Pila scutata* from Peninsular Malaysia and Singapore.

Source (by country)	Locality	Accession No.	Date collected	Remarks
<b>Peninsular Malaysia</b>				
Abdullah & Samsuddin (2005)	Sungai Chini, Pahang			
Annandale (1921)	Penang; Larut, Perak; Selangor; Malacca			
ANSP	Selangor State: Kampong Pandan, Kuala Lumpur	ANSP388823	1965	
ANSP	Selangor State: Sungei Langat	ANSP388824	1965	
ANSP	Selangor State, Kampong Pandan, Kuala Lumpur	ANSP388825	1965	
ANSP	Selangor State, Kampong Pandan, Kuala Lumpur	ANSP388827	1965	
ANSP	Johore, Batu Anam and Muar	ANSP388828	1965	
ANSP	Negeri Sembilan State, Seremban	ANSP388829	1965	
ANSP	Malacca State, Batu Berendam	ANSP388830	1965	
ANSP	Negeri Sembilan State, Kampong Bongek	ANSP388831	1965	
ANSP	Kuala Lumpur, Military Airport	ANSP439086	1966	
ANSP		ANSP50550		From the collection of George Tyron, who died in 1888 (P. Callomon, pers. comm.)
Chan (1998a,b); Chan	Gunung Panjang, about 6.5 km E of Ipoh City, Kinta District, Perak;		1990s	

Source (by country)	Locality	Accession No.	Date collected	Remarks
Sow-Yan, pers. comm.	Gunung Rapat (Chinese Temple, Sam Poh Tong), about 5 km SSE of Ipoh City, Kinta District, Perak; Gunung Rapat (Chinese Temple, Kwan Yin Tong), about 7.0 km SSE of Ipoh City, Kinta District, Perak; Gunung Lang (Chinese Temple, 18 Law Han), about 3 km NNE of Ipoh City, Kinta District, Perak; Gua Teluk Melaka, Lenggong, Perak; Bukit Chondong (known fossil locality), Beseri Mukim, Perlis; Near Kampung Tebing Tinggi, Setul Formation, Wang Bintong Mukim, Perlis; Bukit Keteri (Twin Peaks), near a cement factory, Chuping Mukim, Perlis			
FMNH	Negeri Sembilan, south of Kuala Pilah on Tampin Rd.	FMNH173443, 173446	1963	
FRIM	Sungai Besar, Selangor			
Ghosh (1929)	Ponds and ditches from Kuala Lumpur			
Johnson (1957)	Lake Chenderoh, Perak		Aug. 1957	
Lim et al. (1978); Boo Liat Lim, pers. comm.	Perlis; Alor Setar, Kedah; Kampung Machang, Kelantan; small ricefield plots in rural areas in Kuala Lumpur; Kampung Beranang, Negri Sembilan; Kampung Alai, Malacca; Tanjung Karang, Kuala Selangor		1970s	
MNHN; de Morgan (1885); Cowie & Héros (2012)	Streams in the Province of Wellesley, in vicinity of Bukit Tambun	MNHN-IM-2000-24514		
NHMUK	Found at high tide limit, N.C.O.'s beach, R.A.A.F. Butterworth	NHMUK.1969.206	June 1968	
NZSI; Nevill (1877)	Penang	NZSI2420, M25079/5		
Palmieri et al. (1979, 1980)	Lakes, ponds, streams in Selangor			
Sykes (1902)	Kelantan			
UMMZ	Malacca, Straits Settlements	UMMZ93142		
UMMZ	Penang, Straits Settlements	UMMZ93183		
UMMZ	Perak, Straits Settlements	UMMZ93190, 93217		
ZMA; van Benthem Jutting (1960)	Ditch about 100 m from the main road between Tandjong Rambutan and Ipoh, Perak; near the hot springs, near Tambun, E. of Ipoh, Perak; Sidney Lake, near Kuala Lumpur, Selangor; rice fields about 6 miles W. of Kuala Pilah, Negri Sembilan.	ZMA40450	28 Jan. 1946	
ZMH	Pulau Pinang, ditch at airport	ZMH28830	11 May 1966	
ZMH	Kuala Lumpur - W military airport	ZMH43359	16 May 1966	

Source (by country)	Locality	Accession No.	Date collected	Remarks
ZMH	Malacca	ZMH43361		From the collection of Otto Semper, who died in 1907 (Coan & Kabat, 2017)
ZMH	Kuala Lumpur old airport	ZMH43364		Record catalogued in 1974
ZMH	Kampong Pedang near Kuala Lumpur	ZMH43371	15 May 1966	
ZRC	Negri Sembilan, ricefield 19 miles from Seremban	ZRC.1989.1948		Record catalogued in 1989
ZRC	Selangor, Kuala Lumpur, Lake	ZRC.1989.354	1 June 1955	
ZRC	Malacca, Kampung Baru Gubah	ZRC.1989.355	4 Nov. 1959	
ZRC	Selangor, Kuala Lumpur, Sydney Lake	ZRC.1989.356-357	14 Dec. 1958	
ZRC	Kedah, Sungai Putat	ZRC.1989.361		Record catalogued in 1989
ZRC	Malacca, Kampung Baru Gubah	ZRC.1989.362-363	4 Apr. 1960	
ZRC	Selangor, Kuala Lumpur, 4 1/2 mile, Ampang Road, tin mine, pool and stream	ZRC.1989.364-367	1 June 1955	
ZRC	Selangor, Kuala Lumpur, University of Malaya, Botany Department, concrete tanks	ZRC.1989.369-369	27 Oct. 1956	
ZRC	Malacca, 4 miles Muar Road, ditch	ZRC.1989.376-377		Record catalogued in 1989
ZRC	Negri Sembilan, Sungai Jempol, Batu Kikir	ZRC.1989.378	22 Dec. 1958	
ZRC	Sri Meyanti (Sri Menanti?), Tebat in nipah	ZRC.1989.463		Record catalogued in 1989
ZRC	Selangor, Kuala Lumpur, 4 1/2 mile, Ampang Road, disused mining pool	ZRC.1989.464-465	1 June 1955	
ZRC	Malacca, Kampung Baru Gubah, ditch	ZRC.1989.536-539		Record catalogued in 1989
ZRC	Johor, 13.5 km from Ayer Hitam to Kluang, 2nd stream	ZRC.MOL.6955	23 May 1991	
<b>Singapore</b>				
ANSP	Fishpond, N. Buona Vista Road	ANSP200814		Record catalogued in 1956

Source (by country)	Locality	Accession No.	Date collected	Remarks
Ghosh (1929)	Ditches along Kim Keat Road			
Ponniah (1962)	University pond, Botany Department tank, North Buona Vista Road pond, 115-6 Clementi Road pond, Jurong Road ponds; 13 mile. Jurong Road ditch; 17 mile. Jurong Road pond; Sungei Whampoe canal; Potong Pasir pond; Lorong Buang Kok ditch; Jalan Kuala Simpang ditch		1962	
ZRC	Potong Pasir	ZRC.1989.1947	2 July 1962	
ZRC	Kian Teck Road	ZRC.1989.1949	23 June 1962	
ZRC	Sungei Whampoa	ZRC.1989.358	28 June 1961	
ZRC	Jurong Road, 11 1/2 miles	ZRC.1989.359-360	28 June 1961	
ZRC	North Buona Vista Road, Fish pond	ZRC.1989.375		Record catalogued in 1989
ZRC	Potong Pasir	ZRC.1989.379-385	27 June 1962	
ZRC	University of Malaya, Botany dept tank	ZRC.1989.386-388	25 May 1962	
ZRC	Buona Vista, pond	ZRC.1989.389-397	11 Nov. 1958	
ZRC	Buona Vista, pond	ZRC.1989.398-399	20 Nov. 1957	
ZRC	Seletar Reservoir	ZRC.1989.400-402	4 Feb. 1981	
ZRC	Braddell Road, Sungei Kallang	ZRC.1989.403	16 May 1962	
ZRC	MacRitchie, Sungei Whampoa	ZRC.1989.404-407	16 May 1962	
ZRC	Yio Chu Kang, 8 miles	ZRC.1989.408-414	25 Aug. 1962	
ZRC	Clementi Road ditch	ZRC.1989.417-419	21 May 1962	
ZRC	Clementi Road pond	ZRC.1989.420-422	21 May 1962	
ZRC	Clementi Road pond	ZRC.1989.423-432		Record catalogued in 1989
ZRC	Jurong Road, 17 3/4 miles	ZRC.1989.425-432	28 May 1962	
ZRC	Jurong Road, pond	ZRC.1989.433-437	23 May 1962	
ZRC	Jurong Road, pond	ZRC.1989.438-445	28 May 1962	
ZRC	Jurong Road, pond	ZRC.1989.446-451	23 May 1962	
ZRC	Jurong Road, 11 miles	ZRC.1989.452-453	20 July 1960	
ZRC	University of Malaya, pond	ZRC.1989.466	15 May 1962	

Source (by country)	Locality	Accession No.	Date collected	Remarks
ZRC	North Buona Vista Road	ZRC.1989.467-481	21 May 1962	
ZRC	Lower Peirce Reservoir Road	ZRC.1990.15654-15671	21 July 1990	
ZRC	Institute of Education	ZRC.1990.16215-223	10 Sep. 1988	
ZRC	Seletar Reservoir, off Mandai Road	ZRC.1990.17176-77	6 Dec. 1990	
ZRC	Tengah Reservoir	ZRC.1990.17452-56	26 Dec. 1990	
ZRC	Lower Peirce	ZRC.1990.674	29 Nov. 1990	
ZRC	Kranji Reservoir, upstream	ZRC.1991.20053-66	31 Aug. 1988	
ZRC	Kranji Reservoir	ZRC.MOL.3094	1 July 1905	
ZRC	Tampines Eco Green Park	ZRC.MOL.3132	2 July 2011	
ZRC	Lower Seletar Close, canal under Lentor Avenue	ZRC.MOL.3135	Oct. 2010	
ZRC	Bukit Batok Nature Reserve Park	ZRC.MOL.6949	7 Nov. 1990	
ZRC	Bukit Batok Nature Reserve	ZRC.MOL.6950	19 Nov. 1990	
ZRC	Pasir Ris Dr 1, canal	ZRC.MOL.5	13 May 2002	
ZRC	Kranji Reservoir	ZRC.MOL.6	7 July 2002	

ANSP, Academy of Natural Sciences, Philadelphia; FMNH, Field Museum, Chicago; FRIM, Forest Research Institute Malaysia, Kuala Lumpur, Malaysia; NHMUK, Natural History Museum, London; NZSI, Zoological Survey of India, National Zoological Collection India; UMMZ, University of Michigan Museum of Zoology, Ann Arbor; ZMA, Zoologisch Museum Amsterdam; ZMH, Zoologisches Museum Hamburg.

Malay terms: sungai/sungei, river; kampung, village.

SUPPLEMENTARY TABLE 2 Primers used to obtain sequences.

Primers	Sequences	References
LCO1490	5' GGTCACAACAAATCATAAAGATATTGG 3'	Folmer et al. (1994)
HCO2198	5' TAAACTTCAGGGTGACCAAAAAATCA 3'	Folmer et al. (1994)
dgLCO1490	5'-TAAACTTCAGGGTGACCAAAARAAYCA-3'	Meyer (2003)
dgHCO2198	5'-GGTCAACAACAAATCATAAAGAYATYGG-3'	Meyer (2003)
jpgHCO2198	5' TAIACYTCIGGRTGICCRAARAAYCA 3'	Geller et al. (2013)
mICOintF	5' GGWACWGGWTGAACWGTWTAYCCYCC 3'	Leray et al. (2013)
16Sar-L	5' CGCCTGTTTATCAAAAACAT 3'	Palumbi et al. (2002)
16Sbr-H	5' CCGGTCTGAACTCAGATCACGT 3'	Palumbi et al. (2002)
Uni16SF	5'- <i>TRACYGTGCDAAAGGTAGC</i> -3'	Zhan et al. (2014)
Uni16SR	5'-YTRRTYCAACATCGAGGTC-3'	Zhan et al. (2014)

SUPPLEMENTARY TABLE 3 Top hits on GenBank for COI and 16S sequences of *Pila* spp.

Identity based on morphology (country)	COI		16S	
	Fragment length	Top Hit on BLAST (fragment cover; % identity)	Fragment length	Top Hit on BLAST (fragment cover; % identity)
<i>Pila scutata</i> (Singapore)	313–657	<i>Pila conica</i> <sup>1</sup> EF514948 (97; 99)	361	<i>P. conica</i> EF519167 (100; 100)
<i>P. scutata</i> (Malaysia)	313–657	<i>P. conica</i> EF514948 (100; 99–100)	361–543	<i>P. conica</i> EF519167 (97–100; 100)
<i>P. scutata</i> Philippines	313–657	<i>P. conica</i> EF514948 (98–100; 99–100)	352–392	<i>P. conica</i> EF519167 (98–99; 100)
<i>P. scutata</i> (Indonesia)	320	<i>P. conica</i> EF514948 (100; 94)	486	<i>P. conica</i> EF519167 (100; 95)
<i>Pila ampullacea</i> (Malaysia)	567	<i>P. conica</i> EU274570 (99; 87)	532	<i>Pila</i> sp. EF519180 (98; 90)
<i>Pila angelica</i> (Malaysia)	657	<i>P. conica</i> EU274570 (95; 88)	NA <sup>2</sup>	NA <sup>2</sup>
<i>Pila gracilis</i> (Malaysia)	626–657	<i>P. ampullacea</i> EU274569 (97; 99)	532	<i>P. ampullacea</i> FJ710218 (91; 99)
<i>P. gracilis</i> (Vietnam)	NA <sup>2</sup>	NA <sup>2</sup>	540	<i>P. ampullacea</i> FJ710218 (90; 99)
<i>Pila virescens</i> (Vietnam)	NA <sup>2</sup>	NA <sup>2</sup>	556	<i>Pila polita</i> <sup>1</sup> FJ710220 (88; 100)

<sup>1</sup>*P. conica* is a junior synonym of *P. scutata*, and *P. polita* is a junior synonym of *P. virescens* (Cowie, 2015).

<sup>2</sup>Barcode was not obtained.

## References

- Abdullah, M. & Samsuddin, F. (2005) Diversity and abundance of aquatic molluscs in Sungai Chini and associated wetlands. In: *Sumber Asli Tasik Chini* (eds Idris, M., Hussin, K. & Mohamad, A.L.), pp. 85–101. Penerbit Universiti Kebangsaan Malaysia, Bangi.
- Annandale, N. (1921) The apple snails of the Malay Peninsula. *Journal of the Federated Malay States Museums*, 10, 193–196.
- Chan, S.-Y. (1998a) A brief collecting trip to Perak, West Malaysia Part Two. *The Papustyla*, 12, 1–2.
- Chan, S.-Y. (1998b) A brief collecting trip to Perak, West Malaysia Part One. *The Papustyla*, 12, 4–5.
- Coan, E.V. & Kabat, A.R. (2017) 2,400 years of malacology, 14th ed., January 27, 2016, 1,443 pp. + 110 pp. [Annex 1 – Book Collations] + 65 pp. [Annex 2 – Küster Collation], 51 pp. [Annex 3 – Journal Collations]. American Malacological Society. Available from: [http://www.malacological.org/2004\\_malacology.html](http://www.malacological.org/2004_malacology.html) [accessed Nov 24 2017].
- Cowie, R.H. (2015) The recent apple snails of Africa and Asia (Mollusca: Gastropoda: Ampullariidae: *Afropomus*, *Forbesopomus*, *Lanistes*, *Pila*, *Saulea*): a nomenclatural and type catalogue. The apple snails of the Americas: addenda and corrigenda. *Zootaxa*, 3940, 1–92.

- Cowie, R.H. & Héros, V. (2012) Annotated catalogue of the types of Ampullariidae (Mollusca, Gastropoda) in the Muséum national d'Histoire naturelle, Paris, with lectotype designations. *Zoosystema*, 34, 793–824.
- de Morgan, J. (1885) Mollusques terrestres & fluviatiles du Royaume de Perak. *Bulletin de la Société Zoologique de France*, 10, 353–428.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R.C. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Geller, J., Meyer, C., Parker, M. & Hawk, H. (2013) Redesign of PCR primers for mitochondrial cytochrome C oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13, 851–861.
- Ghosh, E. (1929) Papers on Malayan aquatic biology: Notes on some freshwater molluscs. *Journal of the Federated Malay States Museums*, 14, 388–396.
- Johnson, D.S. (1957) Size-variation in the edible pond-snail. *Malayan Nature Journal*, 12, 185–186.
- Meyer, C.P. (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, 79, 401–459.
- Nevill, G. (1877) *Catalogue of Mollusca in the Indian Museum, Calcutta*. Government Central Press, Calcutta.
- Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T. & Machida, R.J. (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, 10, 34.
- Lim, B.L., Yap, L.F., Krishnansamy, M., Ramachandran, P. & Mansor, S. (1978) Freshwater snail consumption and angiostrongyliasis in Malaya. *Tropical and Geographical Medicine*, 30, 241–246.
- Palmieri, M.D., Palmieri, J.R. & Sullivan, J.T. (1979) The natural diet of *Pila scutata* (Mousson, 1848), a common Malaysian prosobranch snail. *Malayan Nature Journal*, 32, 343–345.
- Palmieri, M.D., Palmieri, J.R. & Sullivan, J.T. (1980) A chemical analysis of the habitat of nine commonly occurring Malaysia freshwater snails. *Malayan Nature Journal*, 34, 1–7.
- Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (2002) *The Simple Fool's Guide To PCR*, ver. 2. University of Hawaii, Honolulu.
- Ponniah, A.J. (1962) Studies on some non-marine aquatic gastropods of Singapore island, with a note on five species collected from Malacca. Honours thesis, National University of Singapore.
- Sykes, E.R. (1902) On a collection of land and fresh water shells from Kelantan, Malay peninsula. *Journal of Malacology*, 9, 60–63.
- van Benthem Jutting, W.S.S. (1960) Some notes on the land and freshwater Mollusca of Malaya. *Basteria*, 24, 10–20.
- Zhan, A., Bailey, S.A., Heath, D.D. & MacIsaac, H.J. (2014) Performance comparison of genetic markers for high-throughput sequencing-based biodiversity assessment in complex communities. *Molecular Ecology Resources*, 14, 1049–1059.