

1 **Supplement to Genner *et al.* Amassing diversity in an ancient lake: evolution of a**
2 **morphologically diverse parthenogenetic gastropod assemblage in Lake Malawi.**
3 **Molecular Ecology (2007) 16, 517–530. doi: 10.1111/j.1365-294X.2006.03171.x**

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5 **Supplementary Online Information 1**

6

7 **Taxonomic notes**

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9 In total thirty-eight endemic species have been described from the lake (Dohrn 1865; Smith
10 1877; Bourguignat 1889; Smith 1893; von Martens 1897), but most have been subsequently
11 synonymized (Crowley *et al.* 1968; Mandahl Barth 1972; Brown 1994). The most recent
12 revision, based on shell and radula morphological characters, recognises three endemic taxa *M.*
13 *polymorpha* (Smith), *M. nodicincta* (Dohrn) and *M. simonsi* (Smith), and two non-endemic taxa
14 *M. tuberculata* (Müller), and *M. virgulata* (Ferrusac) (Eldblom & Kristensen 2003).
15 Mitochondrial DNA sequences indicate that *M. nodicincta* and *M. simonsi* are synonymous with
16 the *M. tuberculata* morph indigenous to Lake Malawi (morph LMN), while individuals
17 identified as *M. virgulata* are synonymous with an invasive morph of *M. tuberculata* (morph
18 LMI) that has more recently colonised Lake Malawi and surrounding water bodies (Genner *et al.*
19 2004; Sorenson *et al.* 2005).

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1 **Supplementary Online Information 2**

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3 **New COI sequences generated for this study**

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Species	Collection site	Year	Collector	Genbank Accession
<i>Melanoides anomala</i>	Kiseru River, Shaba Province, Congo	1988	AP	AY958727
<i>Melanoides anomala</i>	Mwati River, Shaba Province, Congo	1988	AP	AY958726
<i>Melanoides polymorpha</i> (MP _A)	Cape Maclear, Lake Malawi	2002	MJG	AY958742, AY958743, AY958753
<i>Melanoides polymorpha</i> (MP _B)	Cape Maclear, Lake Malawi	2002	MJG	AY958730, AY958757
<i>Melanoides polymorpha</i> (MP _C)	Cape Maclear, Lake Malawi	2002	MJG	AY958728, AY958729
<i>Melanoides polymorpha</i> (MP _D)	Cape Maclear, Lake Malawi	2002	MJG	AY958744, AY958745, AY958746, AY958747
<i>Melanoides polymorpha</i> (MP _E)	Cape Maclear, Lake Malawi	2002	MJG	AY958731, AY958732
<i>Melanoides polymorpha</i> (MP _F)	Nkhata Bay, Lake Malawi	2002	MJG	AY958749, AY958755
<i>Melanoides polymorpha</i> (MP _G)	Nkhata Bay, Lake Malawi	2002	MJG	AY958734, AY958748
<i>Melanoides polymorpha</i> (MP _H)	Nkhata Bay, Lake Malawi	2002	MJG	AY958737, AY958750
<i>Melanoides polymorpha</i> (MP _I)	Nkhata Bay, Lake Malawi	2002	MJG	AY958738, AY958739, AY958740, AY958751, AY958756
<i>Melanoides polymorpha</i> (MP _J)	Nkhata Bay, Lake Malawi	2002	MJG	AY958741, AY958752
<i>Melanoides polymorpha</i>	Nkhata Bay, Lake Malawi	2002	MJG	AY958733, AY958735, AY958736, AY958754
<i>Melanoides admirabilis</i>	Malagarasi River, Tanzania	2002	AC	AY958725
<i>Melanoides mweruensis</i>	Kilwa Island, Lake Mweru	2005	DAJ	DQ995481
<i>Melanoides imitatrix</i>	Isokwe Island, Lake Mweru	2005	DAJ	DQ995479, DQ995480
<i>Tarebia granifera</i> (TGR1)	Coral Gables, Miami, Florida	2003	TR	AY958764
<i>Tarebia granifera</i> (TGR2)	Chinese Gardens, Singapore	2003	MJG	AY958761
<i>Tarebia granifera</i> (TGR3)	Lower Selatar Reservoir, Singapore	2003	MJG	AY958760, AY958762
<i>Tarebia granifera</i> (TGR4)	Napier Road, Singapore	2003	MJG	AY958763
<i>Thiara cf. granum</i>	Aquarium population, Germany.	2003	BP	AY958759
<i>Thiara scabra</i>	Lower Selatar Reservoir, Singapore	2003	MJG	AY958758

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Collectors: MJG = Martin Genner, AP=Andrzej Piechocki, AC = Andrew Cohen, TR = Timothy Rawlings, BP = Bernd Poßeckert. DAJ=Domino Joyce .

1 **Supplementary Online Information 3**

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3 **Previously published COI sequences used in phylogenetic analyses**

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Species	Collection site	Collector	Year	Genbank Accession	Reference
<i>Thiaridae sensu stricto</i>					
<i>Melanoides polymorpha</i> (TRU)	Mwaya, Tanzania: Lake Malawi	-	-	AY791916	Sorenson <i>et al.</i> 2005
<i>Melanoides polymorpha</i> (PUB)	Kiwira, Tanzania: Lake Malawi	-	-	AY791919	Sorenson <i>et al.</i> 2005
<i>Melanoides polymorpha</i> (POL)	Njisi, Tanzania: Lake Malawi	-	-	AY791922	Sorenson <i>et al.</i> 2005
<i>Melanoides polymorpha</i> (NYA/POL)	River Songwe, Tanzania: Lake Malawi	-	-	AY791925	Sorenson <i>et al.</i> 2005
<i>Melanoides polymorpha</i> (NYA)	Lake Malawi Tanzania: Kiwira,	-	-	AY791926	Sorenson <i>et al.</i> 2005
<i>Melanoides polymorpha</i> (MAG)	Lake Malawi Tanzania: Mwaya,	-	-	AY791929	Sorenson <i>et al.</i> 2005
<i>Melanoides tuberculata</i> (TAI)	Itungi, Lake Malawi	-	-	AY791909	Sorenson <i>et al.</i> 2005
<i>Melanoides tuberculata</i> (MAC)	Southern Lake Malawi	-	-	AY791912	Sorenson <i>et al.</i> 2005
<i>Melanoides tuberculata</i> (KLV)	Lake Victoria, Kenya	-	-	AY791913	Sorenson <i>et al.</i> 2005
<i>Melanoides admirabilis</i>	Malagarasi River, Tanzania	EM	1995	AY213151	West and Michel 2000; Michel 2004
<i>Melanoides admirabilis</i>	Lake Tanganyika Basin	-	-	AY456561	Wilson <i>et al.</i> 2004
<i>Melanoides tuberculata</i>	Malawi	-	-	AY456562, AY456563	Wilson <i>et al.</i> 2004
<i>Melanoides tuberculata</i>	Philippines	-	-	AY456564	Wilson <i>et al.</i> 2004
<i>Melanoides tuberculata</i>	French Polynesia	-	1998	AF236071, AF236072	Myers <i>et al.</i> 2000
<i>Melanoides tuberculata</i> (BAN)	Pathun Tani, Bangkok, Thailand	JPP	1998	AY575971	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (ISR)	Ilan, Israel	JH	1993	AY575994	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LIS)	Lisuli Dam, Chikwawa, Malawi	MJG	2002	AY575983	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMI ₁)	Senga Bay, Lake Malawi	MJG	2002	AY575992, AY575988	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMI ₂)	Cape Maclear, Lake Malawi	MJG	2002	AY575985, AY575986	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMI ₃)	Mkungula, Lake Malombe, Malawi	MJG	2002	AY575987	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMI ₄)	Makakola, Lake Malawi	EM	1999	AY575980	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMN ₁)	Nkhata Bay, Lake Malawi	MJG	2002	AY575993, AY575998	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMN ₂)	Cape Maclear, Lake Malawi	MJG	2002	AY575984	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LMN ₃)	Mkungula, Lake Malombe, Malawi	MJG	2002	AY575990, AY575991	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LSD)	Lower Selatar (Storm Drain), Singapore	MJG	2003	AY575976	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LSR)	Lower Selatar Reservoir, Singapore	MJG	2003	AY575978	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (LSS)	Lower Selatar Reservoir, Singapore	MJG	2003	AY575989	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (MAY)	Mayotte Island, Comores Archipelago	JPP	2000	AY575997	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (NAP)	Napier Road, Singapore	MJG	2003	AY575972	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (PAN)	Pandan Reservoir, Singapore	MJG	2003	AY575974, AY575975	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (SOM)	Eil Spring, Somalia	TF	1992	AY575973	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (SRI)	Kandalama Lake, Dambulla, Sri Lanka	NDV	2003	AY575981	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (USR)	Upper Selatar Reservoir, Singapore	MJG	2003	AY575977, AY575979	Genner <i>et al.</i> 2004
<i>Melanoides tuberculata</i> (VIC)	Mwanza Gulf, Lake Victoria	FW	2002	AY575995, AY575996	Genner <i>et al.</i> 2004
<i>Tarebia granifera</i>	French Polynesia	-	1998	AF236073	Myers <i>et al.</i> 2000
<i>Thiara amarula</i>	Mayotte Island, Comores Archipelago	JPP	2000	AY575997	Genner <i>et al.</i> 2004
Other taxa					
<i>Lavigeria grandis</i>	Lake Tanganyika	EM	-	AY213193	Michel 2004
<i>Hydrobia glyca</i>	-	-	-	AF467640	Wilke & Pfenninger 2002

6

Collectors: JPP = Jean-Pierre Pointier, MJG = Martin Genner, TF = Tim Fison (samples provided by David Brown, Natural History Museum, London), NDV = Nicole de Voogd, FW = Frans Witte, EM = Ellinor Michel, JH = Joseph Heller.

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1 **Supplementary Online Information 4**

3 **Calibration of nodes for estimating divergence times**

5 Fossil freshwater cerithioids have an extensive fossil record. Despite this, confident assessment
6 of the systematic affinities of fossil cerithioids for determining the minimum age of nodes can be
7 difficult. This is because: 1) prevailing standards of shell character description and delimitation
8 remain rudimentary (Papadopoulos *et al.* 2004); consequently identification of detailed shell
9 homologues remains uncertain across a wide range of taxonomic levels; 2) some clades show
10 extensive inter- and intra-specific variability in shell morphology, including whorl shape and
11 ornamentation; 3) most fossil taxa have not been studied in detail since their descriptions, long
12 before deep divergence among the crown freshwater cerithioid clades was indicated (Lydeard *et*
13 *al.* 2002); 4) a significant number of taxa have been described from incomplete or poorly
14 preserved specimens and re-evaluation is dependent upon recovery of systematically informative
15 material. Consequently the minimum dates on nodes discussed below are conservative and have
16 been established using only records we consider systematically justifiable.

18 1) Earliest Thiaridae sensu stricto. *Melanotarebia distincta* (Zittel) from freshwater deposits of
19 Middle Eocene, Lutetian age (49.0-41.3 Ma) from Western Hungary (Bandel & Kowalke 1997)
20 has an adult shell that strongly resembles those of extant thiarids such as *Melanoides*. It has an
21 evenly and minutely wrinkled embryonic shell, today restricted to brooding taxa (Riedel 1993),
22 succeeded by a protoconch II terminated by a sinusigera. Comparative studies of extant
23 Thiaridae *sensu stricto* reveal that *Melanotarebia* larval shells have a combination of character
24 states shared by Recent thiarid genera and place it as the earliest confirmed member of the
25 Thiaridae *sensu stricto*.

1

2 2) Earliest *Tarebia*. The most ancient well-dated records of *Tarebia* occur in Southeast Asia and
3 include *Tarebia martini* (Oostinghe) from the Kalibiuk Formation, Early Pliocene (N18/N19) of
4 Java (Skwarko and Sufiati 1994), dated to 5.60-4.18 Ma (Berggren *et al.* 1995). We re-examined
5 taxa currently placed in *Tarebia* from the Late Eocene (Priabonian) of England including
6 *Tarebia acuta* (J. Sowerby) (Glibert 1962) and conclude that they do not belong to this genus
7 and require systematic revision.

8

9 3) Earliest *Thiara scabra*. Currently the oldest records identified as *Thiara scabra* (Müller)
10 include those described in detail by Oostinghe (1935), see van Benthem Jutting (1956) and
11 Skwarko & Sufiati (1994), from the Early to Mid Pliocene Sondian molluscan stage: N19/N20 of
12 Java (Shuto 1975) dated to 4.18-2.8 Ma (Bolli and Saunders 1985; Berggren *et al.* 1995).

13

14 4) Earliest *Melanooides*. Species previously referred to *Melanooides*, with varying degrees of
15 certainty, have been described from deposits as old as the Early Cretaceous (Kase 1984;
16 Hamilton-Bruce *et al.* 2004), but worldwide we have found no pre-Miocene records that can be
17 identified to this genus with confidence. Stratigraphically earlier records can either be discounted
18 after evaluation of published morphological information, are systematically uninterpretable due
19 to poor or incomplete preservation, or require detailed systematic re-evaluation, for example
20 *Melanooides nystii* (Nyst) from the Early Oligocene (Rupelian) of NW Europe (Glibert 1962).
21 Two species from freshwater deposits of the Lake Turkana Basin of Kenya, Early Miocene (18-
22 17 Ma) (Van Damme & Pickford 2003) may represent the oldest *Melanooides*. The next confident
23 and well-dated record appears to be *M. verniersi* Van Damme and Pickford from the Mohari
24 Formation, Semliki Plain, D.R. Congo (Middle Miocene: 12.5-10.5 Ma), and following this there
25 is a fairly extensive record of the genus in East Africa from Late Miocene (7-5 Ma) through to

1 the Recent (Van Damme and Pickford 2003). Older records from Eurasia cannot be substantiated
2 at present.

3

4 5) Earliest member of the *Melanooides admirabilis* clade. We reassessed specimens from Malawi
5 referred to *M. nodicincta* (Dohrn) and dated to Unit 3A, Chiwondo Beds (3.3-2.3 Ma) by van
6 Damme & Pickford (2003). With Recent African species of *Melanooides*, these specimens share
7 the following features with only *M. admirabilis* (Smith): a) strong, straight to gently abaxially
8 arched, markedly opisthocline ribs on adult whorls; b) growth increments run parallel to axial
9 ribs; c) strong shoulder groove below a strong subsutural spiral cord; d) depressed area above the
10 lower suture in which the axial ribs die out, and e) strong spiral ornament on base of whorl.
11 Consequently, we tentatively consider this putative taxon and *M. admirabilis* to be sister species,
12 thus providing a date for the earliest taxon comprising the *M. admirabilis* clade, rather than
13 identifying it with extant *M. nodicincta* of Lake Malawi.

14

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