

## Unravelling some Kinki earthworms (Annelida: Oligochaeta: Megadrili: Megascolecidae) - Part II

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**Abstract.** *Metaphire tanbode* sp. nov. is found in rice paddy in Kinki plain at Lake Biwa and *Amyntas yamade* sp. nov. is from Hira range to the West. *M. tanbode* belongs to the *M. hilgendorfi* / *A. tokioensis* species-complex, while montane *A. yamade* is comparable to both *Amyntas aeruginosus*-group and *Duplodicrodrilus schmardae*-group. Genetic barcoding (mtDNA COI) via types is attempted. Taxonomic ‘housekeeping’ requires replacement of invalid homonyms: e.g. *Pheretima montana* Ishizuka, 1999 (non type-species *P. montana* Kinberg, 1867) is renamed *Amyntas nonmontanus*; others are *A. nonsilvestris*, *A. noninvisus*, *A. nonmonticolus* and *A. nonsetosus*, noms. et combs. novae. Thus *Pheretima* Kinberg, 1867 s. stricto remains unrecorded from Japan while prior *Amyntas* Kinberg, 1867, and its derivative *Metaphire* Sims & Easton, 1972, are abundant and diverse. Family and generic level definition and placement of Oriental pheretimoids are restated for the benefit of current workers and for novice field-ecologists. Surveys of below-ground biodiversity of rice paddy in Lake Biwa is compared to more natural habitats around Lake Pedder in Western Tasmanian Wilderness Area, and co-incidentally, both have 21 recorded earthworm species. Thus claims from various countries of less than six species per location are contraindicated by thorough eco-taxonomic methods yielding more representative results.

### INTRODUCTION

*Sine systemate chaos* – Quote from title page to “Das Tierreich – Vermes” (Michaelsen, 1900).

Of the ~6,000 terrestrial megadrile taxa currently known (plus ~4,000 microdriles), the pheretimoids (*Pheretima* auct.) comprise 13 genera and ~940 valid species from ca. 1,400 nominal taxa (Blakemore, 2007a). This is nearly double the 746 nominal (sub-)species reviewed by Sims and Easton (1972) and is raised considerably from Michaelsen’s (1900) list of just 167 valid *Pheretima* species. They form a large and important group of Oriental earthworms with a few peregrine members spread worldwide, especially in the tropics (see Blakemore, 2002, 2008)

Rather than belonging in the exemplary genus *Pheretima* Kinberg, 1867, most pheretimoids now default to the prior *Amyntas* (et *Amyntas* praeocc.) Kinberg, 1867 or belong in its derivative, *Metaphire* Sims & Easton, 1972 that may not be monophyletic, as its original authors well realized. However, throughout its 250 years, conventional Linnaean zoological taxonomy has evolved to culminate in the current ICZN (1999) which, in its Preface to the Fourth Edition, accepts that traditional nomenclature “may be equally applied to paraphyletic as to monophyletic groups”. New ge-

nera were established mainly for taxonomic ‘convenience’ although the *Metaphire* non-superficial male pores are an obvious apomorphic development from superficial male pores of *Amyntas*. Degree of development of non-superficial pores is irrelevant for generic placement (cf. Gates, 1982; James *et al.*, 2005; James, 2005) except newly for *Duplodicrodrilus* Blakemore, 2008 (Japanese type *Megascolex schmardae* Horst, 1883) that has the greatest derivation in its male pore structures. Only secondarily is development of manicate intestinal caeca accorded taxonomic significance since these function as culture ‘nurseries’ for microbial digestive symbionts and are thus adaptive and peripheral to key reproductive characteristics. This accords with the systematics of Michaelsen (1900) and Stephenson (1930), unlike Gates (1972, 1982) who, while being a constant critic of their ‘Classical System’, also deliberately ignored contemporaneous revisions of Sims and Easton (1972) and Easton (1979) for a decade.

Under the terms of ICZN (1999: Art. 57.2), junior primary homonyms are objectively and permanently invalid, but junior secondary homonyms are only treated as invalid whilst considered congeneric (Art. 59) and may be reinstated, with any replacement name proposed after 1960 entering their synonymy (Art. 59.4). A consideration for replacement of junior homonyms, ICZN

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(1999: Art. 23.3.6), is whether “...when treated as a junior synonym; it may be used as the valid name of a taxon **by an author** who considers the synonymy to be erroneous...” (my bolding); and a criterion of ICZN (1999: Art 60.2.1) states: “Such a name can be retained as a valid name in place of a junior homonym only as long as it is regarded as a synonym of the latter.”

This is the current situation in Japan. It has recently been brought up in conversation, in manuscript submission and in web searches (noted below) that the synonymy of several homonyms are considered erroneous by some contemporary workers, not least by their original author who has had 10 years notice to correct his numerous nomenclatural lapsae. Instances of retrograde taxonomy, reversing revisionary decisions of Sims and Easton (1972), Easton (1981) and Blakemore (2003, 2007a, 2008), that revert to superseded taxonomy are two recent publications, viz. Minamitani *et al.* (2007) and Minamitani *et al.* (2009)] wherein there is an attempt to reinstate abandoned names

such as the “*Pheretima aokii*” synonym of prior *Metaphire soulensis* (Kobayashi, 1938). The invalid nomen nudum “*Pheretima montivaga*” as noted by Blakemore (2003) continues to surface from time to time, most recently on a Texas A & M University website (<http://www.globalnames.org>) and a database from Thomson-Reuters ION facility: ([http://www.organismnames.com /details.htm](http://www.organismnames.com/details.htm)), (both accessed 12.III.2010).

In such situations it is perhaps precautionary and expedient to provide available and potentially valid replacements as a matter of due process in the interests of ICZN (<http://iczn.org/>) stated aims for “Standards, sense and stability for animal names in science”.

Regarding eco-taxonomic sampling, the purpose for correctly and uniquely naming taxa, Japan as with any other region has the following contingencies:

Contingency chart of biodiversity sampling reliability

Case	Ecological sampling	Taxonomic treatment	Results
1	+	+	Representative data
2	+	–	Under/over-estimate
3	–	+	Under/over-estimate
4	–	–	Unrepresentative data

+, Good; -, Poor

Summary results of modest earthworm survey of paddy fields around Lake Biwa (Blakemore, 2007b, 2010; Blakemore *et al.*, 2010; Blakemore & Kupriyanova, 2010), are comparable with an earlier week-long Lake Pedder earthworm survey (conducted by RJB in 1996 – see Blakemore, 2000a, b). In this context, the frequent under-estimation of earthworm biodiversity in agro-ecological bio-assessments is briefly discussed.

## MATERIALS AND METHODS

Classification follows the convention and methodology style of Blakemore (2000a, b), as modified slightly by Blakemore (2002, 2008) that allows for organic variability in natural members

of a taxonomic entity. Nomenclature complies with ICZN (1999) code.

DNA extraction, amplification and sequencing methodology follows that given in Blakemore *et al.* (2010) and in Blakemore and Kupriyanova (2010) and this new data, as presented in Appendix 1a, 1b, will be submitted simultaneously to the GenBank online facility [<http://www.ncbi.nlm.nih.gov/genbank/>].

## RESULTS

### Substitute names

New replacement names are provided for permanently invalid homonyms, each assuming the same type-specimens, under the requirements of ICZN (1999).

*Etymology.* Latinized names are all derived by preposition of ‘non-’ meaning ‘not’.

*Amyntas nonmontanus* Blakemore **nom. et comb. nov.** pro *Pheretima montana* Ishizuka, 1999: 103 (Holotype NSMT-An 263) non *Pheretima montana* Kinberg, 1867: 102 – the type-species of the genus *Pheretima*. Under ICZN (1999: Art. 57.2) *P. montana* Ishizuka, 1999 is an objectively and permanently invalid junior primary homonym. A substitute name was not previously provided for Ishizuka’s taxon as it was considered a synonym (ICZN, 1999: Art. 60). In some, but not all, distributed reprints of Ishizuka (1999) the name “*montana*” is crossed out and another name (“*montivaga*”) inked in pen. This does not constitute a valid published nomenclatural act under the code (ICZN, 1999: Arts. 8, 9). In a subsequent paper by Ishizuka (2001: 12, 14, 92) – that appears to contravene ICZN (1999: Art. 11.4) requirement for consistent binomial application – the name “*Pheretima montivaga* Ishizuka, 1999” appears as a non-explicit nomen nudum (ICZN, 1999: Arts. 13, 16) apparently for this taxon. Transfer to *Amyntas* in synonymy with *Amyntas fuscatus* (Goto and Hatai, 1898) had provisionally removed this primary homonym from use (see ICZN, 1999: Arts. 23.3.5; 53.3; 60), in the interests of nomenclatural sense and stability, as noted above, a valid replacement name is now provided.

*Amyntas nonsilvestris* Blakemore **nom. et comb. nov.** pro *Pheretima silvestris* Ishizuka, 2000: 18 (Holotype NSMT-An 298) a permanently invalid primary homonym, non *Pheretima silvestris* Michaelsen, 1923 (= *Amyntas silvestris*). Under ICZN (1999: Arts. 57.2, 60) this junior primary homonym is permanently invalid but it was not previously replaced as synonyms exist. Currently it is held as one of the 40 synonyms of cosmopolitan *Amyntas corticis* (Kinberg, 1867), as with *P. invista* and *P. setosa*. The synonymy of the *A. corticis*-group has recently been questioned by some authors in manuscript and in preliminary unpublished molecular analyses (as published on GenBank online facility), thus a replacement name is considered warranted pending thorough revision of the *A. corticis* species-complex.

*Amyntas noninvisus* Blakemore **nom. et comb. nov.** pro *Pheretima invisus* Ishizuka, 2000: 189 (Holotype NSMT-An 326) a permanently invalid primary homonym, non *Pheretima invisus* Cognetti, 1913 (= *Metapheretima invisus*). “*Invisus/invisa*” in Latin most usually means “hateful”. Blakemore (2003) stated this junior primary homonym “is permanently invalid under ICZN (1999: Arts. 57.2, 60) but is not replaced as synonyms exist for it”. In order to presage possible restoration from *A. corticis* synonymy, it is now replaced, as with *P. silvestris* and *P. setosa*.

*Amyntas nonmonticolus* Blakemore **nom. et comb. nov.** pro *Pheretima monticola* Ishizuka, 2000: 191 (with segments miscounted in figs. 65-66) (Holotype NSMT-An 328) a permanently invalid primary homonym, non *Pheretima monticola* Beddard, 1912 (= *Polypheretima monticola* from the Philippines). Blakemore (2003) listed this taxon as a

junior synonym of *Amyntas conformis* (Ishizuka, 2000: 182) but mistakenly stated that “an invalid manuscript name “*montivaga*” was sometimes supplanted over this name” – cf. *P. montana* above. *Amyntas conformis* possibly belongs in synonymy with *A. yamizoyamensis* (Ohfuchi, 1957) that is currently held under *Amyntas micronarius* (Goto and Hatai, 1898) pending revision. Editors of the Global Names Index (at least) incorrectly list the homonym as a valid name ([http://www.globalnames.org/name\\_strings?page=726&search\\_term=ns%3APHE\\*](http://www.globalnames.org/name_strings?page=726&search_term=ns%3APHE*) accessed 12. III. 2010), thus a replacement name is necessary to avoid undue confusion.

*Amyntas nonsetosus* Blakemore **nom. et comb. nov.** pro *Pheretima setosa* Ishizuka *et al.* in Ishizuka, Shishikura & Imajima, 2000: 188, figs. 25-33 (Holotype NSMT-AN-342) a permanently invalid primary homonym, non *Pheretima setosa* Cognetti, 1908 [= *Metaphire sieboldi* (Horst, 1883)]. Under ICZN (1999: Arts. 57.2, 60) this junior primary homonym is permanently invalid but was not previously replaced as synonyms existed. Currently held as one of the 40 junior synonyms of *Amyntas corticis*, as with *P. silvestris* and *P. invista* although some author(s) apparently disagree with all these synonymies and attempt restoration (vide supra).

*Amyntas palarvus* (Blakemore, 2003) **comb. nov.** pro ‘*Pheretima palarva*’ was a replacement name of junior secondary and primary homonyms under ICZN (1999: Arts. 53.3, 57.2, 60.3, 67.8, 72.7) for *Pheretima parvula* Ishizuka *et al.*, 2000: 186 [non *Perichata parvula* Goto and Hatai, 1898 (?= *Amyntas gracilis*); nec *Pheretima parvula* Ohfuchi, 1956 (= *Metaphire parvula*)]. This finally removes the only single, albeit tentative, *Pheretima* taxon remaining in Japan that is itself a probable new parthenogenetic synonym of either *Amyntas micronarius* (Goto and Hatai, 1889) or *A. carnosus* (Goto and Hatai, 1889), or of some other taxon.

## DESCRIPTIONS OF NEW SPECIES

### *Metaphire tanbode* Blakemore sp. nov.

(Fig. 1)

*Type material.* Preliminary rice paddy survey of Tanakami region near Kurotsu, southern Otsu-shi, Shiga-ken, Kinki, Japan sample stations #1–3, leg. RJB, all deposited with initial Accession No.: Misc. Invert. FY2009–13 in Lake Biwa Museum (hereafter, LBM) initially tagged as “*A. shigai* sp. nov.?”. Holotype (H) ex samples from Kurotsu, 5-chôme (type locality), Otsu-shi, 35°1’N, 135°51’E, elev. ~80 m, sketched, dissected and providing tissue for DNA extraction: LBM 138000094; Paratypes (P1–P6) with same collection data as H but including pooled specimens

from three closely adjacent sites, only P1, a posterior amputee, dissected: LBM 1380000095 (6 specimens); P7 same collection data but from yet another paddy in the same vicinity, male field figured and a tissue sample taken from posterior for comparative DNA analysis: LBM1380000096.

*Etymology.* Japanese (genderless) noun phrase in apposition meaning “from the paddy”.

*Diagnosis.* Pheretimoid with spermathecal pores in 6/7/8, non-superficial male pores, manicate caeca and genital markings as a single, mid-ventral sucker from 17/18 to setal arc of 18 that lacks secondary papillae (as found in *M. hilgendorfi* spp-group members). Spermathecal diverticula are particularly elongate, more than twice the ampulla length.

*Distribution.* Restricted to type-locality. It is surprising that this species, which is so patently a wanderer, appears to be not yet more frequently and more widely recorded. Such a restricted distribution is more often characteristic of an introduced species, unless its superficial similarity to *M. hilgendorfi* (Michalsen, 1892) has caused oversight.

*External features.* Holotype and all paratypes appear mature. H, P1 and P7 dissected. Body circular in section throughout. Slight brown pigmentation mostly in anterior dorsum. Lengths ca. 70–100 mm by 2–3 mm [H, 92; P1, 60 (posterior amputee); P2, 95; P3, 85, P4–6, 70–90 and P7, 100 mm]. Segments ca. 100 (H, 92). Setae approx. 55 on 12 in H; or ca. 50–60 per segment thereafter. Prostomium open epilobous. First dorsal pore in 12/13 (H). Spermathecal pores small ca. 0.4U apart in 6/7/8. Clitellum 14–16. Female pore single, central on 14. Male pores ca. 0.4U apart on slightly raised tumid mounds: primary pores on small porophores withdrawn into slight invaginations (i.e. just classable as “non-superficial”). In H the male pores are sunk in small pouches, but in P7 they protrude slightly and are seen to be on the apices of small mounds protruding from puckered lips (see Fig. 1). Ge-

nital markings as moderately large tumid sucker-like pad in 17/18 extending to setal arc of 18. Corresponding puckered area mid-ventrally on 8 construed as an artefact of copulation and not an actual marking (it is also missing from P3, P6). Variations: P2 is abnormal as male pores are on 17lhs and 18rhs, spermathecal pores are in 6/7/8rhs but only 6/7lhs and genital markings are in 16/17 as well as in 17/18; P3 lacks 18rhs male pore and spermathecal pores are 6/7/8lhs but only 7/8rhs.

*Internal anatomy.* Septa 8/9/10 absent, 10/11 thin. Gizzard after 7/8. Last heart in 13. Nephridia meric in forests, absent from spermathecal ducts. Male organs holandric with testis in 10 and 11 and seminal vesicles in 10 and 11 (possibly slightly in 12 but not clear). Spermathecae in 7 and 8: usual ampulla with diverticulum having long stalk and cayenne-chili or paprika-shaped bulb, all charged with sperm in H but are uncharged and displaced to 9lhs in P3. Ovaries quite small with funnels in 13; ovisacs absent from 14. Prostates glandular with long, muscular U-shaped duct in 18 that is joined at its junction with gland by vas deferens. Copulatory pouch not pronounced internally. A sessile glandular pad under ventral nerve cord in 17–18 corresponds with genital marking. Intestine from ½15. Typhosole simple, dorsal ridge from about 20. Intestinal caeca from about 27, manicate, e.g., with four ‘fingers’ on rhs in H.

*Ingesta.* Organic silt and decayed plant stems and organic debris (paddy soil).

*Behaviour.* Found wandering on road surface between paddy fields early in the morning (~8:00AM) thus probably stranded on the hard asphalt – to which no earthworm is yet accustomed – after a night’s excursion on the surface. There was evidence of recent sex (puckered marks and spermathecae charged), thus it may be surmised both that copulation occurs above ground and that only semi-permanent burrow

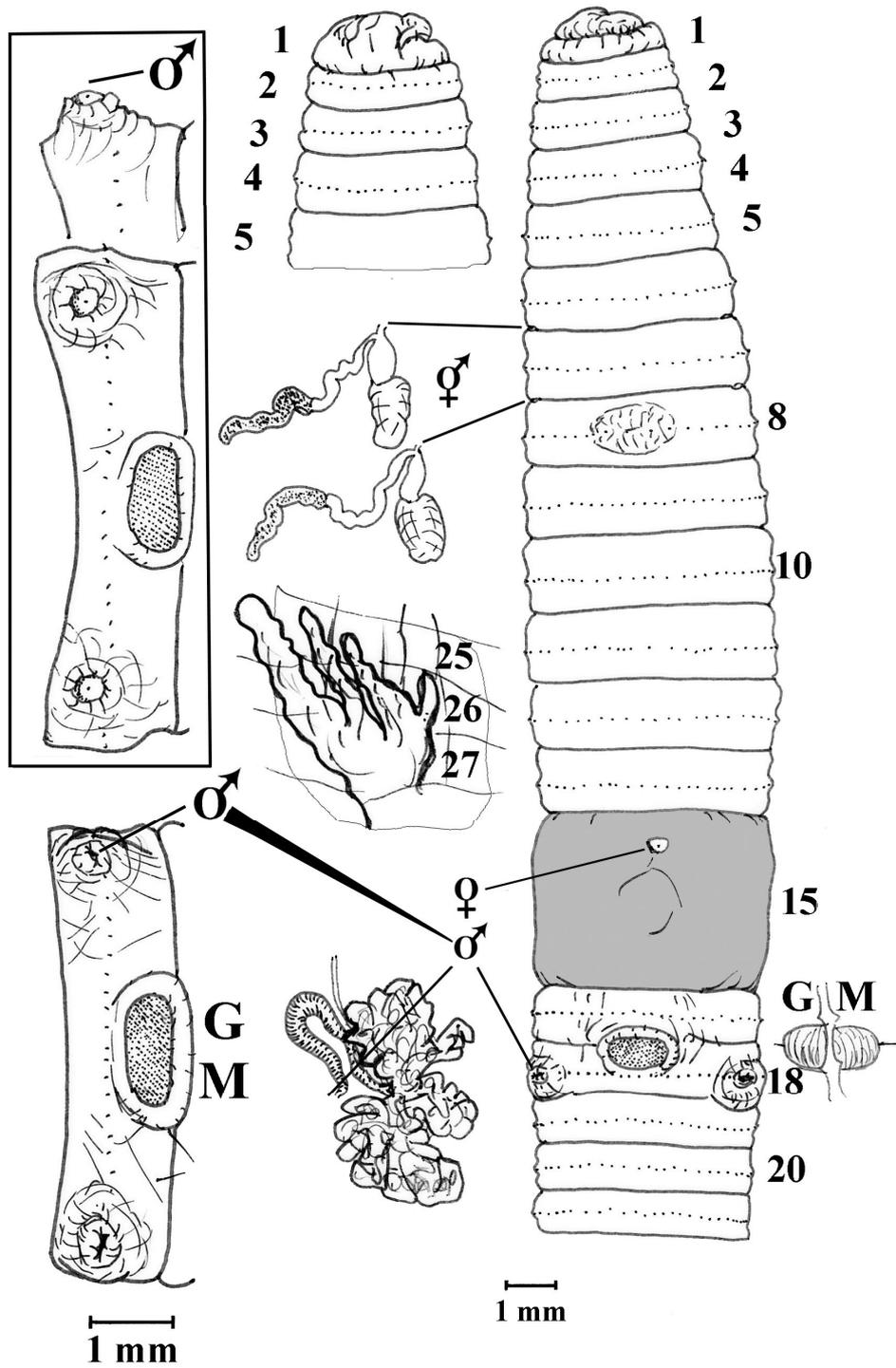


Figure 1. *Metaphire tanbode*. Anterior dorsum and ventrum of H with spermatheca, prostate, genital marking gland and caecum shown *in situ*; the male field of H is enlarged and that for P7 is boxed for comparison

systems are maintained, or that the ability to follow a return trail to a particular burrow is unreliable.

*Genetics.* Small tissue samples taken from non-essential posterior segments of H and P7 for DNA extraction, amplification and sequencing (results in Appendix 1a and GenBank).

*Parasites and predators.* Numerous nematodes were found in coelom near prostates in H and some are stored in a separate vial in the sample jar.

*Ecology/Species associations.* Little is yet known of its ecology except that it appears to survive in periodically cultivated and waterlogged paddy soils. Other species found on the same collecting trip (Table 1 below) were: Monilogastridae: *Drawida eda* Blakemore, 2010 (as described in accompanying paper – Part 1); Ocnerodrilidae: *Eukerria saltensis*; Megascolecidae: *Amyntas megascolioides*; *A. corticis*; *A. gracilis*; *A. hupeiensis*; *Metaphire hilgendorfi* spp-complex; Lumbricidae: *Eisenia japonica*; *Dendrodrilus rubidus* and *Helodrilus hachiojii*. Some species, in mixed assemblages, were in remarkably high numbers in the moist paddy soils; several leeches were also found and two of their cocoons collected.

*Remarks.* Summary of the current taxon is: spermathecal pores in 6/7/8, intestinal caeca manicate – as is frequently found in Japanese/Korean species – plus a single, central, genital-marking pad presetally in 17/18,18 only. In Easton (1981) this taxon keys out as either a component of what is now the *Metaphire hilgendorfi* / *Amyntas tokioensis* species complex, or as '*Pheretima koellikeri*'. Approximately 74 valid species have spermathecae in 6/7/8 (Blakemore, unpubl.); of these, about 21 are known to have manicate caeca plus having genital markings, when present, sometimes unpaired (but excluding such members of the *M. hilgendorfi*/*A. tokioensis* that are known to have paired markings or central markings comprising numerous papillae). Comparable regional species and most are poorly described and require extensive revision, in chronological order, are:

*Amyntas vittatus* (Goto & Hatai, 1898: 74) inadequately described with glandular genital markings equatorial in 7 and 8 (that Goto & Hatai mistook for spermathecal pores) and often lacking male pores. It has striped coloration (pers. obs.).

*Amyntas parvicystis* (Goto & Hatai, 1899: 18) inadequately described with glandular genital markings paired anteriorly in 7 and 8 that (Goto & Hatai mistook for spermathecal pores) plus paired markings median to male pores (when present).

*Amyntas? yunoshimensis* (Hatai, 1930: 655, fig. 4) has papillated markings midventrally in 8 and 18, similar to those in *Metaphire hilgendorfi* proper, and in *M. glandularis* (Goto & Hatai, 1899: 18, fig. 9) where the equivalent markings are shown to be in 7 and 17/18. Its spermathecae and male pores were defective.

*Amyntas tappensis* (Ohfuchi, 1935: 409) with synonyms as per Blakemore (2003, 2005, 2007a) including ?*Amyntas sanchongensis* Hong & James, 2001 that although said to be similar to sympatric *Amyntas jiriensis* (Song & Paik, 1971: 193) that is itself most likely a junior synonym of *A. tokioensis* (Beddard, 1892), if not synonymous to *A. tappensis*, possibly qualifies for *Metaphire* due to its probable non-superficial male pores.

*Metaphire servina* (Hatai & Ohfuchi, 1937: 1) [?praeocc. Hatai, 1924] with genital markings small, paired, median to male pores equatorially on 18.

*Amyntas gomejimensis* (Ohfuchi, 1937: 18) that was stated by Ohfuchi (1937: 19) to resemble *Pheretima servinus* Hatai & Ohfuchi, 1937 (= *Metaphire servina*) in all characters except its lack of genital markings; thus, because no fully mature specimens were found [hence it is difficult to understand how Ohfuchi (1937: 20) could describe the clitellum in 14–16], it is possibly in synonymy of that taxon, or some other prior taxon.

*Metaphire soulensis* (Kobayashi, 1938: 131), revived by Blakemore (2003) from unlikely synonymy in *M. yamadai* (Hatai, 1930) by Easton (1981); this taxon is believed to have genital markings median to spermathecal pores in 7 and 8 and within its copulatory pouches. A synonym is *Pheretima aokii* Ishizuka, 1999.

*Amyntas gucheonensis* (Song & Paik, 1970: 106) has paired genital patches in the neighborhood of male pores medially and anteriorly close to the setal line of 18.

*Metaphire geomunensis* (Hong & James, 2001: 82) from Korea described with "male pores at tips of conical porophores" (= penes?) and with genital papillae centered between male pores and in paired groups presetally in 7 near spermathecae. With respect to genital papillae, it is said to be similar to *A. alveolatus* Hong & James, 2001: 81 [that is a junior synonym of *Amyntas kanrazanus incretus* (Kobayashi, 1937: 343)] and to *A. yongshilensis* Hong & James, 2001: 80 [that is a probable junior synonym of *A. k. kanrazanus* (Kobayashi, 1937: 340)].

*Amyntas songnisanensis* Hong & Lee, 2001: 284 from Korea described with genital markings as slightly elevated, circular spots on segments 8–19 variably with none or up to 18 per segment. However, the possibility that these are at least partly parasitic artifacts would

account for their variation and for the thinning or reduction of the clitellum ventrally where these occur. Thus the so-called uniqueness of the unlikely “saddle-shaped” clitellum would be explained and the possibility aired of synonymy with *Amyntas multimaculatus* Hong & Lee, 2001 that has only slight quantitative differences.

*Amyntas ephippiatus* Hong & Lee, 2001: 286 from Korea that has multiple genital markings and can thus be excluded from consideration, although it is probably a junior synonym candidate from several of the prior taxa listed above.

The current taxon complies with Sims & Easton’s (1972: 238) now outmoded *glandularis*-group. In addition to *Metaphire glandularis*, this group had comprised: *Metaphire levis* (Goto & Hatai, 1899: 20) that has spermathecal pores in 6/7/8 surrounded by small papillae with glands internally but typically lacking male pores (thus it is not known how Sims & Easton could reliably transfer this taxon to *Metaphire*) and possibly it is in synonymy of *A. vittatus*, etc.; also *Metaphire servina* and *Metaphire soulensis* as noted above, and *Metaphire vesiculata* (Goto & Hatai, 1899: 21, figs. 13–15) with its current synonyms from Blakemore (2003, 2007a) that all typically lack genital markings.

Similarly to the parasitic artifacts already noted for several *Drawida* taxa (Blakemore & Kupriyanova, 2010) that do not represent primary identifiers, the puckered ‘markings’ in segment 8 are considered artefactual due to copulation. These patches resemble those (RJB pers. obs.) found in *Amyntas agrestis* (Goto & Hatai, 1899) and in *Metaphire hataii* (Ohfuchi, 1937), for example. Henceforth, such dark or puckered patches, as with any parasitic artifacts, are not to be considered as genital markings proper in morphological keys and analyses, merely as identification support indicators.

***Amyntas yamade* Blakemore sp. nov.**

(Fig. 2)

*Type material.* Holotype: (H) Mt. Bunagatake (highest peak in the Hira Mts.), Hirotoni valley (type locality), Kitahira, Shiga-chô (now Kitahira, Otsu-shi), Shiga-ken, Japan, ca. 35°15’N, 135°53’E, elev. 990 m, 26.IX.1993, leg. Shigekazu

Uchida; mature specimen sketched, dissected and donor for DNA tissue sample: LBM1390000091. Paratype: (P) Yoichitani valley, Kase, Kutsukimura (now Kutsuki-Kase, Takashima-shi), Shiga-ken, Japan, elev. 470 m, 22.V.1996, leg. S. Uchida; mature, dissected: LBM1380000092.

*Additional material examined.* One specimen (S), same collection data as P; an undissected, ac clitellate, sub-adult: LBM1380000093.

*Etymology.* Japanese noun-phrase in apposition meaning “from the mountain(s)”.

*Diagnosis.* Pheretimid with spermathecal pores in 7/8/9, superficial male pores on 18, manicate caeca and no markings except for flat dishes around (infolded) male field.

*External features.* Body robust, rounded with much secondary annulation (not shown in figure), tapering to posterior. Numerous gregarine parasitic cysts visible through cuticle. Pigmentation dark but setal arcs paler giving slight striped appearance, also preservative (EtOH?) stained yellow-brown with the distinctive ‘wormy’ odour typical for such specimens. Size 150 by ca. 6–8 mm (H and P), 130 mm (S). Segments 129 (H), 112 (P). Setae 60–70 on 12 (H). Prostomium open epilobous. First dorsal pore 12/13. Spermathecal pores widely paired (ca. 0.4U apart) in 7/8/9. Clitellum 14–16. Female pore central in 14. Male pores opposed in deeply infolded longitudinal trough, each in centre of large elongate or circular dish (in H). Trough not so pronounced in P and not manifest in S. The actual pores although opposed are wide apart (ca. 0.4U) and slightly gaping, but are classifiable as superficial.

*Internal anatomy:* Pharyngeal mass pronounced in 4. Septa around gizzard aborted (seemingly 9/10/11 in H or 8/9/10 in P). My notes do not clarify whether last hearts are in 12 or 13, but typically they are in the latter. Nephridia meroic forests, not present on spermathecal ducts. Spermathecae in 8 and 9 each have large ampulla on short duct with diverticulum same length as duct and ampulla combined. The ampullae are flat-

tened and the kinked termina of the diverticula have iridescent sheen (i.e., apparently charged with semen). Male organs holandric with testes in 10 and 11 and seminal vesicles extensive in 10, 11 and 12. Ovaries and oviducts are small in 13; ovisacs not noted. Prostates are multi-lobed glandular on thick duct that, although wider at exit, does not terminate in a noticeable copulatory pouch. Intestine commences in 16 and the intestinal caeca are manicate with three to five lobes, the larger of which is somewhat incised.

*Ingesta.* Mainly woody and organic debris (detritivour).

*Parasites and predators.* *Monocystis* gregarines abundant internally in H, and nematodes also present: there appears to be one sort in the coelom and another form in the seminal vesicles (stored in vials in sample jar).

*Behaviour.* Nothing yet known on these preserved specimens; although pigmentation and gut contents suggest the species inhabits the superficial soil litter layers.

*Genetics.* Tissue samples from posterior of H taken for DNA analysis (see Appendix 1b).

*Ecology/Species associations.* Having a heavy parasite burden is perhaps characteristic of an established species that has had time to acquire an extensive complement array. Nothing is yet known of details of its ecology except that it is montane. Species found in the same collection series, but not necessarily from the mountains (all identified by RJB) are: 1-7-1-03 No 3-2 (1999) – *Bimastos parvus* (Eisen, 1874); 1-7-1-C1 No. 3-5 & No. 3-2 (1996) – *Metaphire hilgendorfi* / *Amyntas tokioensis* spp-complex; plus several other immature specimens.

*Remarks.* In Easton (1981) this taxon does not key out, but comes closest to *Amyntas robustus* (Perrier, 1872) that is differentiated on its genital markings, or to cosmopolitans *Metaphire californica* (Kinberg, 1867) and *Duplodicrodrilus schmardae* (Horst, 1883) both possibly originally from

Japan that differ, not least, in their non-superficial male pores. Outside Japan/Korea, only about 18 previously known taxa have the combined characteristics of spermathecae in 7/8/9 and manicate caecae (Blakemore, unpubl.). Those lacking genital markings, as here, are:

*Amyntas digitatus* and *A. jampeanus* both from Indonesia by Benham (1896) that have different biometry. *Metaphire musica* (Horst, 1883) from Java, Indonesia living in pandanus trees is larger in size: up to 570 mm long by 48 mm wide, and *Amyntas dangi* (Thai, 1984) from Vietnam is also larger at >300mm long.

In Sims & Easton (1972) the *schmardae*-group, apart from synonyms, only contains *Metaphire paeta* (Gates, 1935) from China that has large genital marking papillae paired in the anterior of 8 and 9. Alternatively, specimens with superficial pores key out to an *A. aeruginosus*-group of nominal taxa that is unreconstructed with regards to intestinal caeca form or other defining characteristics. This latter group includes *Amyntas robustus* and *Amyntas aspergillum* (Perrier, 1872), both having genital markings and simple caeca. As this species is clearly different to these taxa, it is reasonable to conclude that it is species new to Japan and probably a native.

## DISCUSSION

Earthworm taxonomy continues on its “chaotic” course – as decried by Fender & McKey-Fender (1990). Especially the composition of family Megascolecidae is yet confused and inconsistent – see Blakemore (1994, 2000a, b, 2005, 2008a, b) for clarity. In contrast, James *et al.* (2005: 1008) claimed:

“We use the definition of the Megascolecidae offered by Jamieson *et al.* (2002), which is supported by a molecular analysis. It is identical to that of Blakemore (2000), the two systems differing greatly regarding definitions of the Acanthodrilidae, and the nonrecognition by Jamieson *et al.* (2002) of the Octochaetidae and Exxidae.”

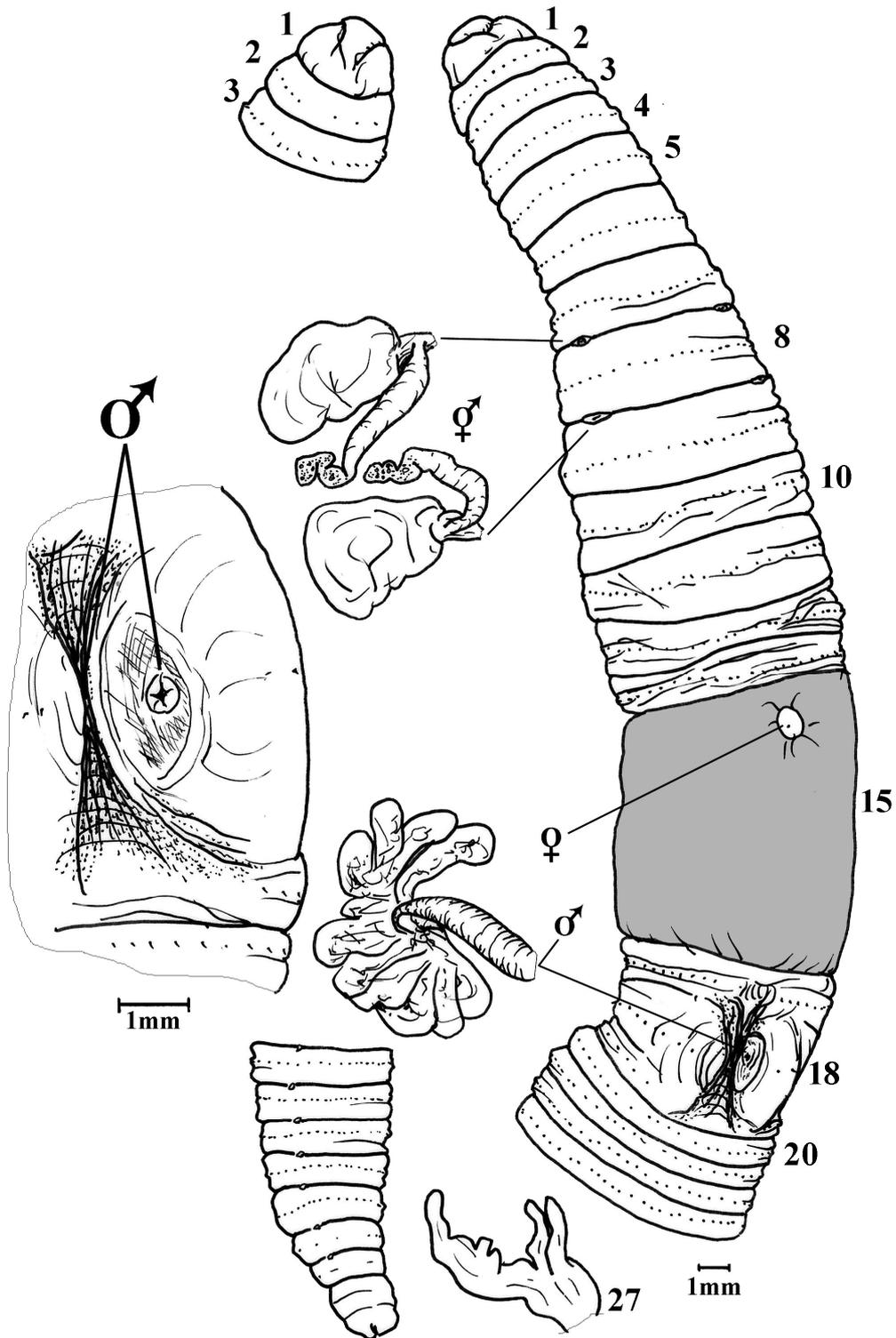


Figure 2. *Amynthus yamade*. Anterior and posterior dorsum and ventrum of H with spermatheca, prostate and caecum shown *in situ*; plus enlargement of 18–20ths male field

This statement is falsifiable for four main reasons: firstly, that by the Principle of Priority, if Blakemore (2000b) is the prior system then that should be accepted; secondly, it is illogical for two systems to be “identical” but also “differing greatly”; thirdly, the fact that Blakemore’s definitions of families are non-Gatesian (Gates, 1959) was ignored. And, finally, if James *et al.* (2005) truly follow the subordinate system then they must reject Acanthodril-IDAE at family level unlike many contemporary authorities including Blakemore (2000b) wherein refined Ocerodrilidae, Acanthodrilidae, Octochaetidae and Megascotocidae *sensu* Blakemore, 2000b (i.e., not some earlier Gatesian concepts) are all acceptable at family level. Moreover, not one type-specimen of a type-species of any type-genus was tested in their cited joint-author paper, some species names were provisional and many of the genus combinations were mistaken; therefore, “molecular analysis” support for their classification is ungrounded and unsound.

As an example of the current confusion, James (2004) apparently unwittingly cited “Acanthodrilidae” in his title, “Megascotocidae” on page 278 yet he was describing *Dichogaster* species that belong in either Benhamiinae and/or prior Octochaetidae; but, by his citing Acanthodril-IDAE, raises the question – just which version?

The taxonomy of Japanese megadriles also languishes in a desperate state and urgently needs a thorough review from the very basics under ICZN (1999) Principles of Priority and Typification (see Easton 1981, Blakemore 2003, 2010). For this reason it has been thought ill-advised until now to add yet further new taxa to this confusion, despite several appearing to be ‘good’ species. In the meantime, several nominal taxa have been added to the national lists, e.g. for Japan, Korea, Philippines, Taiwan and China, without addressing the many underlying historical problems of the regional faunal nomenclature. A reason to recommence biodiversity and ecotaxonomic studies in Japan in the current work is the present opportunity to employ objective DNA barcoding of unique type specimens to help seek

resolution. It is recommended that wherever possible such barcoding be adopted as a standard routine for all new earthworm species from the Orient (and elsewhere) simultaneously with their morphological/ecological description.

On a wider issue, that of the genus *Metaphire* possibly not being monophyletic, this was recognized early on by its authors and subsequently. Sims and Easton (1972: 214) realized the difficulty in placement of several component taxa when they established *Metaphire* mainly for taxonomic ‘convenience’ to provide “smaller, more manageable groups”. However, its male pores in copulatory pouches are obviously a derivative from the superficial male pores as definitive of prior *Amyntas* thus its retention has phylogenetic merit within each of its species groups, regardless of whether acquired synchronistically. Compare this to Gates (1982: 38, 52) who, while knowingly ignoring Sims & Easton’s revision, yet accepted their premise that invagination of genital pores is a secondary development, he nevertheless makes a false distinction of male pores as either “invaginate” or “superficial, i.e., **non invaginate**”.

This key phrase in “**non-invaginate**”, is bolded to emphasize an important point that Gates, as with James *et al.* (2005) and James (2005) who follow this argument, make a fundamental mistake: It is illogical and makes no phylogenetic sense to have secondarily “invaginate” male pores as the primary state when the opposite is the case: e.g., from Blakemore (2000b that follows Michaelsen, 1900, 1907), male pores are either “superficial or **non-superficial**”, just as setae are either lumbricine or non-lumbricine, nephridia are holoic or non-holoic, and prostates are either tubular or non-tubular. The second couplet in each case being derivative. This distinction is even more valid when the current ‘default’ genus is *Amyntas* with superficial male pores, rather than *Pheretima* or *Metaphire* that, by original designation of type-species, both have **non-superficial** male pores. Degree of development from the primitive superficial pores in species with non-superficial male pores remains irrelevant for

generic placement except newly for *Duplodicrodrilus* Blakemore, 2008 that has enormous, ever-sizable, intromittent organs. The issue of parthenogenetic degradation of species, and the effect of this on male pore presence and form, is discussed at length by Gates (1972, 1982) and by Blakemore (2003, 2008) where there is some concordance of authoritative views.

Re-iterating for the benefit of current or future workers who may try to comprehend retrogressive publications such as when James *et al.* (2005) on page 1013 state:

“Gates (1975, p. 7) wrote, “Presence or absence of copulatory chambers is too vague. The really important character is whether the male pores are superficial or invaginate. In the latter case, whether in slight transverse slits or much deeper spaces still confined to the parietes or whether thick-walled copulatory chambers deeply penetrating into coelomic cavity (cf Gates 1972, p. 150)”. and “We support following the suggestion of Gates (1975) to better characterize the status of various types of non-superficial male pores. For now we support restricting *Metaphire* to those species distinguishable from *Pheretima* only by the absence of nephridia from the spermathecal ducts (Sims and Easton 1972).”

James (2005: 130, 137) expresses a similar perspective. Yet this proposition is clearly preposterous as it would exclude the type-species of the genus – i.e., *Metaphire javanica* (Kinberg, 1867) – from membership, contrary to the basic essence of Typification under the ICZN code and as clearly explained by Blakemore (2002, 2003, 2005).

Failure to appreciate the basic taxonomic nature of pheretimoid genera allowed these authors to misconstrue the correct placement of species whereby James *et al.* (2005) described seven “new *Amyntas*” from Taiwan; of these, *Amyntas chaishanensis*, *A. hengchunensis*, *A. kaopingensis*, *A. ailiaoensis* and *A. huangi* belonged in *Metaphire*. Furthermore, all seven species were found to be synonymous:- *A. huangi* to *Metaphire houletti* (Perrier, 1872), *A. chaishanensis* to *M. formosae* (Michaelsen, 1922), *A.*

*kaopingensis* to *M. paiwana*, *A. ailiaoensis* to *M. feijani*, and *A. monsoonus* to *A. tungpuensis*. This synonymy (from re-inspection of types) was by Tsai *et al.* (2006 - <http://gra103.aca.ntu.edu.tw/gdoc/95/D93B41001a.pdf>) although *A. monsoonus* is probably closer to *A. carnosus* (Goto & Hatai, 1899). Moreover, *M. hengchunensis* is in synonymy, or at best a sub-species, of *M. formosae* or *M. paiwana* (according to Chang *et al.*, 2008 – <http://www.ncbi.nlm.nih.gov/pubmed/18809504>) and *A. nanrenensis* is yet another synonym of the *A. corticis* spp-group (pers. obs. and Appendix 2). This information was already passed to the authors before publication in a referee’s report by the current author dated 8<sup>th</sup> January, 2004 which, for the sake of clarity, is attached unadulterated in Appendix 2. Moreover, review of all Taiwan taxa including new species records was already in manuscript form innocently circulated to several Taiwanese authors for comment from 19<sup>th</sup> May, 2004 but which remained unpublished, due to referee and editor delays, until Blakemore *et al.* (2006).

It thus seems that James *et al.* (2005) were wrong – at family, genus, and species levels – and they also failed to adequately prove their arguments with molecular techniques by completely ignoring analysis of types for inexplicable reasons (cf. Blakemore *et al.*, 2010; Blakemore & Kupriyanova, 2010). There is the similar débâcle of the *Pheretima* (*Ph.*) *urceolata* (Horst, 1893) species-group in the Philippines flagged by Blakemore (2010), of *A. carnosus* in Korea and Taiwan (Blakemore, in prep.), and of *Pheretima* (*Ph.*) *darnleiensis* (Fletcher, 1886) that gains two further synonyms in *Pheretima pugnatoris* and *P. tabukensis* **syns. novae** both by Hong and James, 2010 from the Philippines as neither name is justified from the sequentially prior nominal taxa as reviewed by Blakemore *et al.* (2007).

Marginality of male pore form divide is exemplified in the present paper, as similarly discussed for *Metaphire paka* Blakemore, 2007 in Blakemore *et al.* (2007); the current two new taxa serving to illustrate this case in point as both are borderline for membership of their respective genera and either could be transferred to the other’s genus. In fact support for reciprocal transfer in the current study is that a lack of genital

markings (and fewer spermathecae) tends to be a characteristic of *Metaphire* species that have intermittent organs and do not require suckers, papillae and setae to help locate and interlock con-copulants. Whereas in *Amyntas* it is almost obligatory to have genital markings to compensate for the superficial nature of the male pores and to ensure they are co-located with the partner's, often multiple pairs of, spermathecal pores (and to protect from parasitic compromise?). Full taxonomic resolution of these two genera depends entirely upon morphological compliance and DNA analysis of the type-specimens of the type-species (see Blakemore 2008a, b; Blakemore *et al.*, 2010) and, without this, discussion of molecular or morphological “non-monophyly” is pointless.

Another misplaced taxon is Taiwanese *Amyntas sexpectatus* Tsai *et al.* in Tsai, Shen & Tsai, 1999 that should be held as *Metaphire sexpectata* (Tsai *et al.*, 1999) **comb nov.** on the basis of its non-superficial male pores. Parenthetically, if one thinks such reallocations or synonymies are trivial then they miss the point of Science which is to refine information and to correct errors – both our own and those of others, whether living or dead – and, moreover, the sooner this occurs the better for responsible service to concerned parties. The challenge for authors is to provide unique keys or DNA barcodes to any supposed new species when they attempt to compile regional checklists and species guides for the use of field workers and contemporary or future students.

In the current study a small tissue sample was taken from a voucher specimen clearly identified by the current author with exemplary *M. californica* and these results are presented in the Appendix 1b and will be submitted simultaneously to GenBank and iBOL.

Returning now, after much largely unnecessary diversion, to the main topic of concern: that of earthworm biodiversity from eco-taxonomic survey. Gradually a clearer picture is forming of the true below-ground biodiversity in a broad range of habitats. Perhaps the greatest diversity was 24 species from a 290ha Samford farm in South-East Queensland surveyed for a period of two years (Blakemore, 1994). Included were native and exotic earthworms, both new and known.

Similarly, earthworm taxa currently revealed from around Lake Biwa are presented in Table 1, and these are compared to the biodiversity at Lake Pedder in Tasmania in Table 2. These two data sets, obtained from about a weeks' survey each, challenge the perpetuated impression that only a few earthworm species occur at most sites; a consequence of either poor survey or poor taxonomy or, not infrequently, both (see contingency chart in Introduction above).

Results of preliminary Lake Biwa, Japan surveys by the current author (on 31<sup>st</sup> January – 2<sup>nd</sup> February, and 1<sup>st</sup> – 5<sup>th</sup> October, 2007) were published by Blakemore (2007b). These revealed a total of more than a dozen species plus several microdriles (e.g. *Branchiura* sp., ?*Tubifex* spp.) and at least three species of freshwater leeches around the lake from approximately 25 sample sites (representative specimens fixed in alcohol were deposited and are on Lake Biwa Museum's register). A subsequent short visit (17<sup>th</sup> – 20<sup>th</sup> June, 2009) unearthed yet more species, including *Drawida eda* Blakemore, 2010 and *M. tanbode* sp. nov. as subjects for the current series of papers.

Thus the baseline biodiversity and distributions of the two dozen or so megadrile plus microdrile (and leech) species may act as indicators to monitor soil pollution/health in various habitats around Lake Biwa (also for Lake Pedder). An interesting result of the Lake Biwa work was unexpected activity of both clitellate (breeding) adults and of juveniles in an unseasonably warm winter, despite snow on the final day of survey in February, 2007 especially at the lakeside Fudogawa site beside a creek under exotic *Eucalyptus* gum trees that originated in Australia.

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**Table 1.** Earthworm species found at Lake Biwa, Shiga-ken, Kinki, Japan

	<b>FAMILIES and Species</b>	<b>Rice paddy</b>	<b>Other habitats</b>
	MONILIGASTRIDAE		
1	“ <i>Drawida</i> cf. <i>barwelli</i> ” = <i>D. eda</i> Blakemore, 2010	+	
2	<i>Drawida hattamimizu</i> Hatai, 1930	+	
3	<i>Drawida japonica</i> (Michaelsen, 1892)	+	
	OCNERODRILIDAE		
4	<i>Eukerria saltensis</i> (Beddard, 1895)	+	+
	MEGASCOLECIDAE s. Blakemore, 2000b		
5	<i>Amyntas corticis</i> (Kinberg, 1867)	+	+
6	<i>Amyntas gracilis</i> (Kinberg, 1867)		
7	<i>Amyntas hupeiensis</i> (Michaelsen, 1895)	+	
8	<i>Amyntas megascolidioides</i> (Goto and Hatai, 1899)	+	+
9	<i>Amyntas robustus</i> (Perrier, 1872)	+	
10	* <i>Amyntas yamade</i> Blakemore, sp. nov.		+
11	<i>Metaphire californica</i> (Kinberg, 1867)		+
12	<i>Metaphire hilgendorfi</i> (Michaelsen, 1892)	+	+
13	* <i>Metaphire tanbode</i> Blakemore, sp. nov.	+	
14	* <i>Metaphire tosaensis</i> (Ohfuchi, 1937)		+
	CRIODRILIDAE		
15	* <i>Biwadrilus bathybates</i> (Stephenson, 1917)		+
	LUMBRICIDAE		
16	<i>Aporrectodea trapezoides</i> (Dugès, 1828)		+
17	<i>Bimastos parvus</i> (Eisen, 1874)		+
18	<i>Dendrodrilus rubidus subrubicundus</i> (Eisen, 1874)		+
19	<i>Dendrodrilus rubidus</i> (Savigny, 1826) sub-sp?	+	+
20	<i>Eisenia japonica</i> (Michaelsen, 1892)	+	+
21	* <i>Helodrilus hachiojii</i> Blakemore, 2007	+	+
	HIRUDINIDAE and MICRODRILI Benham, 1890		
	Leeches several unidentified spp. + many microdrile spp.	+	+

\*Denotes supposed wholly endemic Japanese spp. *Biwadrilus bathybates* is aquatic.

**Appendix 1a. Raw data mt DNA COI results for *Metaphire tanbode* sp. nov.**

[Small letters mean single strands, capital letters are from double strands].

Code: LK192-193 W5 = *M. tanbode* P7

TcataaagatattggaactctatatttttaggtatctgagctggtataattGGAGCAGGGATAAGACTACTTATTTCGAATTGAGCTAAGACAACC  
TGGATCCTTCCTAGGAAGTGATCAACTTTATAATACAATTGTAACGGCTCACGCATTCTTGATAATTTTTTTTCTTG  
TAATACCCGTATTTATTGGGGGATTTGGAACTGGTTACTACCCCTTATACTAGGGGCACCAGATATAGCATTTC  
ACGACTCAATAATATAAGATTTTGGTTACTACCCCTTCCCTTATTTTATTAGTATCTTCAGCAGCTGTAGAAAAAG  
GTGCAGGGACAGGGTGAACAGTGTATCCACCATTGCAAGAAATATTGCACATGCTGGCCCTCCGTAGACCTAG  
CAATTTTTTCTCTCATTAGCCGGTGCATCATCAATTCTAGGTGCAATTAATTTTATTACTACAGTAATCAATATrC  
GATGGTCTGGACTACGCTTAGAGCGAATCCCGTTATTGTATGAGCAGTTGTAATTACTGTAGTTCTCCTACTTCTA  
TCTCTACCTGTACTAGCCGGTGTATTACAATACTACTAACAGATCGAAACCTAAATACATCCTTCTTTGATCCTGC  
TGGAGGGGGAGATCCGATTCTATATCAACACTTATTCTGATT

GenBank Blast comparisons: *Metaphire feijani* COI 84%, *Amyntas vittatus* COI 86%.

**Table 2.** Earthworm species found at Lake Pedder, Tasmania

	<b>FAMILIES and Species</b>	<b>Garden or path</b>	<b>Natural habitat</b>
	MEGASCOLECIDAE s. Blakemore, 2000b		
1	<i>Vesiculodrilus borealis</i> Blakemore, 2000		+
2	<i>Vesiculodrilus ventralis</i> Blakemore, 2000		+
3	<i>Diporochaeta diadema</i> Blakemore, 2000		+
4	<i>Diporochaeta gordonii</i> Blakemore, 2000		+
5	<i>Diporochaeta lacustris</i> Blakemore, 2000		+
6	<i>Diporochaeta rubertumula</i> Blakemore, 2000		+
7	<i>Diporochaeta setosa</i> Blakemore, 2000		+
8	<i>Perionychella dilwynnia</i> (Spencer, 1895)		+
9	<i>Perionychella variegogata</i> nom. nov. Blakemore, 2010		+
10	<i>Hypolimnus pedderensis</i> gen. et comb. nov. Blakemore, 2000		+*
11	<i>Notoscolex pilus</i> Blakemore, 1997 (syn. <i>Notoscolex dinephrus</i> Blakemore, 2000)		+
12	<i>Aporodrilus dombrovskisi</i> Blakemore, 2000		+
13	<i>Aporodrilus monogynus</i> Blakemore, 2000		+
14	<i>Aporodrilus rubicundus</i> Blakemore, 2000		+
15	<i>Gastrodrilus driesseni</i> Blakemore, 2000		+
16	<i>Anisochaeta clavi</i> Blakemore, 2000		+
17	* <i>Anisochaeta dorsalis</i> (Fletcher, 1887)	+	
	LUMBRICIDAE		
18	* <i>Aporrectodea caliginosa</i> (Savigny, 1826)	+	
19	* <i>Eiseniella tetraedra</i> (Savigny, 1826)	+	+
20	* <i>Allolobophoridaella eiseni</i> (Levinsen, 1884)	+	
21	* <i>Octolasion cyaneum</i> (Savigny, 1826)	+	+
	HIRUDINIDAE and MICRODRILI Benham, 1890		
	Some leeches and several microdriles described in MS only		+

\* Denotes exotic/introduced species for Tasmania. *Hypolimnus pedderensis* is extinct under IUCN red data book reports.

**Appendix 1b. Raw data mt DNA COI results for cf. *M. californica*.**

[Small letters mean single strands, capital letters are from double strands].

Code: LK172-173 W8 = *Metaphire californica* Lake Biwa sample.

atattggaaccttATATTTTATTCTAGGAATCTGAGCAGGGATAATCGGAGCAGGGATAAGATTACTTATTTCGCATCGAAC  
 TAAGACAGCCTGGATCATTCCCTTGGAAGAGACCAACTATATAATACAATTGTGACAGCACACGCATTTCTAATAAT  
 TTTCTTTCTGGTGATACCAGTATTTATTGGGGGATTTGGAAACTGACTTCTCCCACTAATGTTAGGAACCCCTGACA  
 TAGCGTTCCCTCGACTAAATAACATAAGATTCTGACTACTGCCACCCTCATTAAATTCTACTAGTTAGATCCGCGGCA  
 GTAGAAAAGGGAGCAGGTACAGGATGAACAGTGTACCCTCCACTAGCTAGAAACATAGCACACGCAGGTCCATCA  
 GTAGATCTTGCAATTTTCTCACTACATTTAGCAGGTGCCTCATCAATTTTGGGGGCCATCAATTTTCATTACCACTGT  
 GATCAACATGCGATGATCAGGCCTACGCCTAGAGCGAATCCCACTATTTCGTATGAGCCGTAGTAATCACTGTAGTA  
 CTCCTTCTACTATCACTTCTGTGCTAGCGGGAGCAATTAATATATTATTAACGGATCGAAACCTAAATACCTCATT  
 CTTCGACCCTGCAGGTGGGGGAGACCAATTCTGTATCAACACC

GenBank Blast comparisons: *Metaphire californica* COI 100%

Unfortunately, other specimens, including Holotypes of *M. tanbode* and *A. yamade*, did not yield sequence results at this time and require re-analysis although age and unknown preservation method (e.g. formalin fixed?) of the latter species, collected 17 yrs earlier, may be problematical. Samples have been sent for re-testing and, if results are successful, this data will be presented freely on GenBank and iBOL websites.

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Supporting online material: Appendix 2 ([http://opuscula.elte.hu/Tomus41\\_2/Blakemqre\\_App2.pdf](http://opuscula.elte.hu/Tomus41_2/Blakemqre_App2.pdf))