Invasive Alien Species *Triops* (Branchiopoda, Notostraca) in Japan and Its Ecological and Economic Impact

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ABSTRACT

The generally-accepted tadpole shrimp (notostracan) classification of Longhurst does not include information on the world richest *Triops* collection in Budapest, Hungary. Besides, his “*Triops granarius*” (an ‘Asian’ species in the broadest sense), which is ranging from Africa through Eurasia and even to Japan, has been regarded as a single cosmopolitan species with wide morphological variations. However, the morphological and molecular analyses conducted by me revealed that it was a hotchpotch of multiple species. If a regional endemic species has been misidentified as a broadly-distributed one (to sum up, this is the most problematic matter of Longhurst’s classification), then any zoogeographical results based on this would be far from the nature; and as such I opted to verify its species definition. Invasive alien species of tadpole shrimp *Triops* were first introduced into rice fields of Japan in the 1910s and have spread around Japan. The expansion of *Triops* has resulted in negative ecological and potential economic impact. Regarding their establishment or extinction patterns, I built a simple mathematical competition model among two self-fertile and one related sexual *Triops* species of Japan, incorporating reproductive interference. Intense reproductive interference drove the competition to sexual exclusion of self-fertile species. Nonetheless, even under intense reproductive interference, the Allee effect (which reduces the population growth rate of sexual species with low density) allowed self-fertile species to survive when the competition started with the lower densities; conversely sexual species to be left alive when the competition started with the higher densities.

Keywords

invasive alien species, mathematical competition model, revised taxonomy, rice field ecology, tadpole shrimp, threat to Japan’s rice crop

1. Introduction

Tadpole shrimp, or the Notostraca, is one of the most morphologically and ecologically primitive groups of extant crustaceans on the planet. The recent classification considers Notostraca as one of orders of the class Branchiopoda under subphylum Crustacea. Branchiopods are small crustaceans inhabiting many of the fresh and saline inland waters around the world, including the Antarctica. They have well adapted to temporary water bodies as most species have eggs resistant to drying, namely “resting eggs”. Their gills are flattened lobes projecting from the bases of the trunk limbs. Branchiopoda comprises fairy shrimp (order Anostraca), tadpole (or shield) shrimp (Notostraca), clam shrimps (Diplostraca; suborders Spinicaudata, Laevicaudata and Cyclestherida), water fleas (Cladocera) and the Devonian *Lepidocaris*. They are mostly small, freshwater animals that feed on plankton and detritus, and sometimes to be predators.

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Tadpole shrimp *Triops* in the wild are found only in temporary pools and puddles after rain, or some rice fields of the world. They are placed taxonomically under a single family Triopsidae (the Carboniferous–the Recent). The scientific study of tadpole shrimp commenced with Frisch (1732), who reported them in Germany. The current tadpole shrimp classification is reviewed by Longhurst (1955) and references therein. Surprisingly, he reduced several hundred species belong to the genus *Triops* into four species only. His distinguishable characters were the pattern of the telsonal armatures, and also the presence or absence of a second maxilla. The latter especially was a differential diagnosis newly produced by him. However, Longhurst's revision does not contain any data on specimens of the world richest *Triops* collection, i.e., Daday Collection in the Hungarian Natural History Museum (Budapest). For this reason, I surveyed at least 20 times as many samples as those by Longhurst.

The pest damage from tadpole shrimps to Japanese rice fields has been obscure. In recent years, however, in southern part of Australia one species of wild tadpole shrimps, which is becoming established in rice paddies, has been reported. The state government has identified it as a pest and has set up a guideline on how to deal with the tadpole shrimps (Stevens, 2013). Based on these facts, from now on, it is important to distinguish between invasive alien and native species of tadpole shrimps also in Japanese rice fields. In this sense, my study presents an aid to solve the problem of non-native tadpole shrimps in rice production.

2. Material and methods

2.1 Material examined

To define the native Japanese species, I used available specimens of tadpole shrimps from the Ural and Irkutsk regions, Buryatia (these are in Russia), Mongolia, Inner Mongolia (P.R. China), and Japan, plus African specimens and information on Indian species, for my morphological and molecular analyses. In particular, the specimens used as the basis for my redescription in the text and Appendix 1 are listed in Appendix 2. Furthermore, when I began the descriptive work, I have been trying to determine whether Longhurst's (1955) review is reliable.

I have gathered as much information as possible about collections in such museums as the Hungarian Natural History Museum; Natural History Museum Vienna (Austria); Natural History Museum 'Grigore Antipa' (Romania); Natural History Museum, Slovak National Museum; Kyoto University Museum (Japan); The University Museum, The University of Tokyo (Japan); National Museum of Nature and Science (Japan); Lake Biwa Museum (Japan); Muséum nationale d'Histoire naturelle (France); Western Australian Museum; Zoological Museum, Zoological Institute of Russian Academy of Sciences; Zoological Museum of Moscow University (Russia); Breslau Zoological Museum (currently Museum of Natural History, University of Wrocław, Poland); Naturalis Biodiversity Center (The Netherlands) and Natural History Museum, Berlin (Germany).

Nomenclatural and bibliographical errors made in his revision by Longhurst (1955) have been corrected herein as much as possible under my responsibility, and emphasis matters are indicated in square brackets (Appendix 1). The Biodiversity Heritage Library covers the worldwide digitised literature since the 18th century on tadpole shrimps, including many of those overlooked by Longhurst. As a result, I have surveyed almost all of the available original papers, including non-English ones, for nearly 290 years since the first report on these animals was published by Frisch (1732) to date.

In compiling my redescription, I have standardised the abdominal segments' count in measurements. Whereas some authors count the terminal segment of abdomen as the last one, I follow Linder's (1952) opinion in regarding it as a telson. Although various measurement standards have been used to determine carapace length (CL), I herein have used the midline length as in my former publications (e.g., Naganawa, 2018).

2.2 Molecular phylogenetic analysis

This study is based on the ethanol-fixed specimens of the *Triops granarius* s.l. (= *sensu lato*) species group collected by me in the field (2 species: GenBank accession nos. LC260169, LC260170, LC260171, LC260172), and specimens in the Hungarian Natural History Museum (6+ species, well-preserved specimens from the 1900s); as well as comparative DNA sequences retrieved from the GenBank (7 *Triops* species: EF521890, GQ144446, HF911372, HF911376, HF911377, JN175223, JX110639, KF979157, KF979167, KF979168, KF979169, KF979170, MF496656 [Sun and Cheng (2019)]), with an outgroup *Lepidurus viridis* Baird, 1850; HF911398).

Specimens for DNA analyses were preserved in 99% ethanol. Genomic DNA was extracted from one trunk limb (= thoracic appendage) using the High Pure PCR Template Preparation Kit, version 20 (Roche). Fragments of a 658-bp section of the cytochrome *c* oxidase gene subunit I (COI) of the mitochondrial DNA (mtDNA) were amplified bidirectionally, using two universal primers: forward LCO1490, 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', and reverse HCO2198, 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al., 1994). Amplification of mtDNA was performed in the total reaction volumes of 25 µl, consisting of 14.4 µl of sterilised mQ-H₂O, 2.5 µl of reaction buffer solution (10× Ex Taq Buffer, Mg²⁺ plus, 20 mM), 2.5 µl of each primer L/H (5 µM), 2.0 µl of dNTP Mixture (2.5 mM each), 1.0 µl of template DNA and 0.1 µl of TaKaRa Ex Taq (5 U/µl) (TaKaRa Ex Taq® RR001A 250 U, Takara Bio Inc., Japan). A thermocycler was run for the polymerase chain reaction (PCR) amplifications, with the following temperature profiles and conditions: a denaturing step at 94 °C for 3 min (1 cycle), followed by 94 °C for 45 s, 50 °C for 1 min 30 s and 72 °C for 2 min (30 cycles), and the final elongation at 72 °C for 4 min (1 cycle). The amplified products were purified with the High Pure PCR Product Purification Kit (Roche), and sequenced on cycle sequencing and capillary sequencers by the Eurofins Genomics K.K. (Tokyo, Japan).

Sequences were edited and contigs assembled in the software program ATGC, version 7.0.2 (GENETYX®) and consensus sequences were constructed afterwards. All obtained DNA sequences were aligned using the Molecular Evolutionary Genetics Analysis Software, version 7.0.20 (MEGA7). All contigs were BLAST searched in the DDBJ/NCBI databases to verify they were not contaminants (i.e., that the sequence was indeed from the taxon of interest). The evolutionary history was inferred using the neighbor-joining (NJ) method (Saitou and Nei, 1987) and the maximum likelihood (ML) method based on the Kimura 2-parameter model (Kimura, 1980). The evolutionary distances were computed using the *p*-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. These evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985) is shown next to the branches.

2.3 Estimation of regional evolution by haplotype network analysis

To estimate regional evolution (genetic diversity and phylogenetic relationships) of the *Triops granarius* s.l. species group, I also conducted phylogenetic analysis with 56 COI sequences of tadpole shrimps (for further information on the retrieved data from GenBank, see Naganawa, 2018). In this case, a Median-joining network (MJN) method was applied with Asian populations (from Siberia and the Far East of Russia, East Asia, including Japan), using the software Network version 10.0.0.0 for analysis of the mitochondrial COI haplotypes.

2.4 Analyses using mathematical competition models

As regards the establishment or extinction patterns for invasive alien *Triops* species, I built two mathematical competition models. One is a resource competition (+ predatory exclusion) model between two self-fertile, alien

species, based on Nimura (1985). And, the other is based on Tilman's (1982) competition model, i.e., a simple mathematical competition model between two self-fertile and one related sexual species of Japan, incorporating reproductive interference. To make the mathematical model more natural, I also assumed environmental fluctuation and the Allee effect, which reduces the population growth rate of sexual species with low density.

3. Revision of Longhurst's classification

3.1 Which *Triops* is the prehistoric naturalised species of Japan?

The taxonomy of the *Triops granarius* s.l. species group from Africa to Asia had been confused over a considerable period of time (e.g., Modak et al., 2018); and especially since Longhurst's (1955) review. As Modak et al. (2018) referred to my latest work (Naganawa, 2018); I also confirmed herein that *Triops sinensis* (Uéno, 1925) is a junior synonym of *Triops granarius* s.str. (Lucas, 1864). Even so, this species is morphologically and molecular genetically different from the one that has been widely recognised from Africa to the Middle East as "*Triops granarius*" (= *Triops granarius* s.l.), on the basis of my recently performed analyses (Naganawa, 2018; Naganawa, personal observation). And therefore, it should be given for the latter such another valid name as '*Triops dybowskii* (Braem, 1893)' [= *Apus numidicus* var. *dybowskii* Braem, 1893], as resurrected below (see Appendix 1).

I recognised the original description of *Apus granarius* by Lucas (1864) is valid zoonomenclaturally (also Mark J. Grygier, personal communication), but the contents with no figures and no data on its type specimen do not provide us any taxonomically effective information as they are. Therefore, I rechecked Gauthier's (1934) drawings of the type specimen (as Lucas') that written as the number of its apodal segments in males is 10 (or '11' as a custom at the time, including the telson), instead of Lucas' (1864). As a result, we can assume that the species which Lucas described from near Peking in 1864 was NOT about a widely distributing species in Eurasia, i.e., *Triops granarius* "long-tailed" (apodal segments: 12–14, Appendix 3); but about another one that lives exclusively in East Asia, including Japan, i.e., *Triops granarius* "short-tailed" (apodal segments: 8–10, ditto). Consequently, two newly-defined species names: ***Triops dybowskii* (Braem, 1893)** and ***Triops granarius* s.str. (Lucas, 1864)** are necessary for us to distinguish them each other. One of the main aims of this review article is to clarify the long-lasting confused taxonomy of the *Triops granarius* s.l. species group, including these two (Appendix 3).

Furthermore, I herein provide an evaluating report as history of confusion existed between *Triops numidicus* (Grube, 1865) and "*Triops granarius*" (e.g., Uéno, 1925, 1927, 1935, 1940; Mark J. Grygier, personal communication), which are first clearly distinguished by me with accurate synonyms in Appendix 1.

3.2 Newly described taxa

Due to space constraints, only the two most important species zoonomenclaturally are listed in the text as below; and the remaining species are summarised in Appendix 1 and 3.

3.2.1 *Triops dadayi* Naganawa & Forró, new species

Type material.—Holotype (Fig. 1, Appendix 3): adult ♂, TL 36.63 mm; CL 22.58 mm [the tip of each furcal filament is lost, but the left and right lengths are almost equal], deposited in the Department of Zoology, the Hungarian Natural History Museum (NHMUS); Daday Phyllopoda Collection (shown as 'Collectio Dadayana Phyllopoda' on the label), accession no. NHMUS D1911-29; I/B-32 [2 individuals in 1 tube; "D1912-29; I/B-32" in Forró and Brtek (1984) is mistyped]. Locality: Mombasa, Kenya [Note: without data on sampling details, probably collected in early 1900s; collector: unknown].

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Paratype: adult ♂, TL 36.65 mm; CL 22.19 mm [the left furcal filament is broken halfway; and therefore, it is about two-thirds in length of the right one. In addition, the posterior edge of the carapace is highly damaged], all other data as for the holotype, stored in the same bottle of the holotype.

Etymology.—The species is named after Daday (Eugen von [derived from his French name: Eugène Daday de Deés] or Jeno von [derived from his Hungarian name: Daday Jenő]; born in Romania, Hungarian zoologist, 1855–1920) who passed away before the completion of monograph on the Notostraca. I offer support and follow his wish, in recognition of his long and enthusiastic dedication to the taxonomy of this animal group. The name consequently is a noun in the genitive singular.

Description.—Male. Dorsal organ depressed and (sub)triangular or trapezoidal. Carapace oval, its length (including the posterior angles) a little greater than its width, more or less arched, usually convex along whole lateral margin to posterior angle. The 18–20 abdominal segments are uncovered by carapace. Apodal (= limbless) segments count 10. Compound eyes close to each other, large and protruded characteristically. Carina quite strong, reaching the concave margin of the shield, lacks spines. Sulcus pentagonal semicircular, but cut not very deep, on the concave margin 26–27 teeth on each side (about 53 in total). Fourth endite of 1st limb usually as long as, or a little longer than carapace. Strong scales on furcal filaments as Asian *Triops* spp.

Female: Unknown.

Distribution.—Known from the type locality only.

Remarks.—Labelled ‘*Apus Braueri*’ by Daday [confirmed by me at the NHMUS; see also Forró and Brtek (1984)]. This new species is described herein under the responsibility of both I and Dr. László Forró (Budapest), in the order of these names. Please cite this species as *Triops dadayi* Naganawa & Forró, 2020, or *Triops dadayi* Naganawa & Forró, 2020 in Naganawa (2020), for all occasions.

Figure 1 (left): *Triops dadayi* Naganawa & Forró, new species (Daday Phyllopoda Collection, accession no. NHMUS D1911-29; I/B-32)

a, Types in tube; b, same (other side); c, holotype ♂ (right one, dorsal view) and paratype ♂ (ventral view), with the original label as ‘*Apus Braueri*’ (undescribed) by Daday; d, anterior part of carapace (holotype, left lateral view). Scale: c = 20 mm; d = 5 mm.

Figure 2 (right): A proposed, NJ bootstrap (1000 replicates) consensus tree for the *Triops granarius* s.l. species group. MJ tree is omitted. Molecular data of *Triops dadayi* is unavailable; it was assigned here on the basis of morphological features (see also Appendix 3)

3.2.2 *Triops granarius* s.str. (Lucas, 1864), emended

Non: *Apus granarius* in G.O. Sars (1899); *Apus granarius* in G.O. Sars (1901); *Apus granarius* in Uéno (1940)

Syn.: *Apus granarius* Lucas, 1864; *Apus granarius* in Simon (1886); *Apus sudanicus* var. *chinensis* Braem, 1893;

Apus numidicus var. *sinensis* Uéno, 1925; *Apus numidicus* var. *sinensis* in Uéno (1927); *Apus granarius* in Gauthier (1934);

Apus numidicus in Uéno (1935) [in part]; *Apus sinensis* in Koba (1938a, b); *Apus sinensis* in Uéno (1940);

Triops granarius in Longhurst (1955) [in part]; *Triops sinensis* in Xue et al. (2010);

Triops sinensis in Naganawa (2018); in Sun and Cheng (2019);

Triops cf. *granarius* (sp.6) from Osaka (Japan) in Naganawa (2018); *Triops granarius* from Japan in Sun and Cheng (2019)

Type.—Holotype: adult ♂, unregistered other than label ‘Type *A. granarius* Lucas’, is deposited in the Muséum nationale d’Histoire naturelle, Paris, France. The type locality labelled “near Paris” is apparently a mistake in labelling; it is most probably ‘near Peking’.

Diagnosis.—Dorsal organ depressed and triangular. Carapace in males round, only very slightly, if at all, longer than width and flattened. Lateral margins usually slightly concave near the posterior angles. Carapace in females elongated, characteristic Buddhist temple bell-shaped. 21–28 (males) or 15–25 (females) abdominal segments are uncovered by carapace. Number of apodal segments 8–10 (males) or 4–7 (females). Carina lacks spines, or several spines on the rear edge in females. Sulcus round (males) or semicircular (females) and with many spines. Fourth endite of 1st limb in males usually a little shorter than carapace, but in females much shorter. Males having strong scales on furcal filaments as in Asian *Triops* spp. Furcal filaments in both sexes are about as long as carapace (including posterior angles).

Type locality.—near Peking, China.

Distribution.—northeast China, western Japan.

Figure 3: A haplotype network chart, indicating a supposed regional evolution of Asian *Triops*, using the software Network version 10.0.0.0 (cf. Fig. 2)

Each mutation not shown in this figure is supplemented here: Missing intermediate haplotype (shown with asterisk; = extinct or unknown population) Hap 3* is separated from Hap 2* by also 14 mutations. Inside the haplotypes Hap 2*, Hap 3*, Hap 4*, Hap 5 and Hap 6, there is one mutation for each.

Remarks.—The species name “*Triops granarius*” has been used for different species by many authors for several couple of decades. I suggest herein it be restricted as ‘*Triops granarius* s.str.’ to the population in East Asia (see also Appendix 1: Remarks for *Triops numidicus*).

One of the most reliable junior synonyms is *Apus sudanicus* var. *chinensis* Braem, 1893. According to his original description, Fritz Braem described it by eight specimens found in the Breslau Zoological Museum (currently Wrocław, Poland). However, I confirmed that the specimens were lost during the Battle of Wrocław on January of 1945 (Jolanta Jurkowska, personal communication).

To sum up, my newly-proposed phylogenetic tree for the *Triops granarius* s.l. species group is as shown in Fig. 2 (using 1000 bootstrap replicates), assigned to eight species (emended for some of them and described in the text or in Appendix 1) in the single genus *Triops*.

4. Supposed regional evolution history of Asian *Triops*

The MJN analysis revealed six haplotypes of Asian *Triops* (Fig. 3). The Siberian-Asian haplotype Hap 5 represents Asian continental *Triops* populations collected from Ural and Irkutsk region of Russia, Mongolia and Inner Mongolia (P.R. China); whereas Hap 6 stands for three populations from Northeastern China (Manchuria) and Japan, and Hap 1 from India. These haplotypes connected to the network through missing intermediate haplotypes with asterisks, i.e., Hap 2*, Hap 3* and Hap 4*, which are most probably extinct species. The Hap 1 from India was separated from Hap 2 by 52 mutations, and Hap 6 from Hap 4* by 25 mutations; but Hap 5 was separated from Hap 3* by only 13 mutations. The missing intermediate haplotypes Hap 2*, Hap 3* and Hap 4* were separated from each other by 14 mutations. Thus, the long branches indicate that Indian Hap 1 and East Asian Hap 6 were separated from each other earlier than the remaining haplotypes of Asia.

5. Analyses using mathematical competition models

5.1 Two *Triops* (self-fertile species) competition model

Nimura (1985) reported a very interesting result on resource competition, including predatory exclusion, among three species, i.e., a brine shrimp *Artemia* (sexual reproductive strain is considered here) and water fleas which are allied faunas of tadpole shrimps, and rotifers in the same water body. We generally tend to think that, at the individual level, even if the animals are predator to each other, smaller individuals in size have a greater chance of being preyed upon by larger ones. Indeed, given the size of each adult: *Artemia* is about 10 mm in length, water flea is 2 mm at best and 0.2 to 0.3 mm for rotifers. If so, each predatory pressure would be *Artemia* < water flea < rotifer; thus, each survival rate would be in the opposite order.

However, this is not the case in real ecosystems. One reason for such is as follows. The true targets in predation are not the adults, but usually the larvae in the early stages of development being in almost the same size; so it doesn't seem to be a difference in body size that determines each survival rate. Furthermore, each reproductive mode of these aquatic animals is: sexual reproduction in *Artemia*; whereas sexual one or parthenogenesis in water fleas, and usually parthenogenesis in rotifers. In other words, the key in the survival competition among the three species is how to build a mature population for reproduction ahead of the others. Nimura's (1985) model shown below accurately describes this fact.

Initial assumptions of calculation are as follows, where birth rate b (individuals per day), age of first birth r (days), and mortality rate of non-natural death p (the case $p = 0$ means natural death only):

1. For the first birth, r days are required; thereafter, each postnatal female releases b individuals of the next generation per day;
2. Moment mortality rate d is proportional to the sixth power of the age in days (this is the average value obtained through a series of preliminary culture experiments); and by the end of life span, 99% of the total population numbers at 0 day of age die a natural death;
3. Mortality rate of non-natural death p ($\approx$ rate of predation) does not change over time;
4. When i days have passed after release, the population number N_{ij} (at j days of age) was calculated using the following formula:

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$$\frac{dN_{ij}}{dt} = -M N_{ij},$$

where $M = d + p$;

5. Birth number N_{ii} (at i days after release) was given by the following equation:

$$N_{ii} = B \sum_{j=1}^{i-r} N_{ij},$$

where $i > r$. The explanation of constant B is omitted here; see Nimura (1985).

In my case study in the southernmost area of Honshu, the main island of Japan (more exactly in rice fields in Shirahama, Wakayama Prefecture), two invasive alien species of tadpole shrimps have been found to be established there. These two species are both self-fertile *Triops*, but there are significant differences about their origins and the estimated periods of intrusion into Japan. Given the species of Australian origin is represented as sp.1, and the species of American (the USA) origin as sp.2; then their ecological characteristics are as follows.

	Australian sp.1	American sp.2
Life span (days)	40	40
Birthrate (b , individuals per day)	15	15
Age of first birth (r , days)	11	16

Considering these, the biggest difference between the two is the speed of sexual maturity. Both species do not require any males to reproduce and the females (actually hermaphroditic) own lay reproducible eggs by self-fertilisation. The former was established in Japan about 30 years ago; whereas the latter more than 100 years ago. The problem is that despite they occupy the same niche; each average body size is not the same at all. There is a big difference, i.e., the latter being only about half of the former. This is most probably due to a dwarfing by self-fertilisation through cumulative reproductions in the same water environment for longer period.

When each population number keeps steady state under the conditions by the reference values shown above, the sizes of the moment mortality rate d were 0.3–0.4 in sp.1 and about 0.25 in sp.2. These rates mean that about 30% of individuals of sp.1 population have been eaten in one day; in the same way, 22% of sp.2 was prey on a daily basis. In other words, if more than 30% of sp.1 has been eaten in a single day, then the population will disappear; such is the case with sp.2 as well; i.e., more than 22% of sp.2 has been eaten, and then its population will disappear. Here, each birthrate b is the same. Looking at the case of natural death only (the case $p = 0$), the growth rate of sp.1 population was much higher than that of sp.2 (each mortality rate up to first birth is less than 0.01%, though). According to this fact, their age (in days) of first birth r may be considered to have a significant impact on their growth rates (simply put, the earlier the age of first birth, the higher the growth rate of population). Given this estimation, the disappearance of sp.2 is mostly caused by much predation under the slow first birth, and NOT caused by the difference in body size. Since the population number has been reduced significantly before the next generations are born, sp.2 population is most probably not being able to maintain themselves.

Figure 4: Case study of rice fields in Shirahama, Japan: a conceptual chart for ‘resource competition (+ predatory exclusion) + reproductive interference’ among three *Triops* (coexisting of two self-fertile and one related sexual species)

Thus, it is much convenient for most population studies of aquatic organisms that Nimura’s (1985) model allows us to exclude from calculations of such variations in growth environment as water temperature, day-night ratio and amount of food resources, because they are common to all of the comparative species in the same water. This model can explain it to some extent that the exclusion of aboriginal sp.2 (American origin) from the rice fields in Shirahama has been accelerated by rapid conversion to newcomer sp.1 (Australian origin). On the other hand, if there are differences in reproductive mode among the comparative species, then it would be not enough to evaluate merely the difference in the rate of maturation of the individuals. In fact, a third sexual *Triops* species coexists in the rice fields in Shirahama. Therefore, another model for verification is necessary as given below.

5.2 Three *Triops* (two self-fertile and one sexual species) competition model

That brings me to the establishment or extinction patterns of coexisting multiple *Triops* species; I built a simple mathematical competition model, based on Tilman’s (1982), between two self-fertile and one related sexual *Triops* species of Japan, incorporating reproductive interference. The calculating scheme is shown in Fig. 4.

For only one species, let the species population density N , birth rate b , mortality rate d ($b > d > 0$) and congestion degree h , then the general logistic equation can be written using differential equations as follows:

$$\frac{dN}{dt} = (b - d)N - hN^2.$$

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Interspecific competition among three species is considered, i.e., two self-fertile, alien species: Australian origin (sp.1) and American origin (sp.2), and one related sexual, Asian native one (sp.3). In resource competition, the population density N_{sp} ($sp = 1, 2$ or 3) of the opponent species increases the congestion degree h_{sp} . The effect of resource competition of sp.1 on sp.2 is indicated as c_{21} , and the effect of sp.2 on sp.1 is as c_{12} . In the same way, between sp.2 and sp.3; and between sp.1 and sp.3 are shown as c_{32} , c_{23} ; and c_{31} , c_{13} , respectively.

The population densities of sp.1, sp.2 and sp.3 are N_1 , N_2 and N_3 in this order, then

$$\begin{aligned}\frac{dN_1}{dt} &= (b_1 - d_1)N_1 - h_1N_1(N_1 + c_{12}N_2 + c_{13}N_3); \\ \frac{dN_2}{dt} &= (b_2 - d_2)N_2 - h_2N_2(N_2 + c_{21}N_1 + c_{23}N_3); \\ \frac{dN_3}{dt} &= (b_3 - d_3)N_3 - h_3N_3(N_3 + c_{31}N_1 + c_{32}N_2).\end{aligned}$$

Computer-based calculations were performed, with the mathematical model algorithms and graphing instructions embedded in the sheet (Fig. 5).

Next, reproductive interference is introduced. In reproductive interference, b_{sp} of females (sp.1, $sp = 1$; sp.2, $sp = 2$) decreases with the relative frequency of males (sp.3) of the opponent species, that is,

$$I(N_1) = \frac{N_1}{N_1 + i_{13}N_3}; I(N_2) = \frac{N_2}{N_2 + i_{23}N_3},$$

where i_{13} and i_{23} are coefficients for the strength with which the males of sp.3 reduce the birth rate b_{sp} (sp.1, $sp = 1$; sp.2, $sp = 2$) respectively. In this case,

Figure 5: A calculating sheet for the mathematical competition model (see the text for further information).

$$\begin{aligned}\frac{dN_1}{dt} &= (I(N_1)b_1 - d_1)N_1 - h_1N_1(N_1 + c_{12}N_2 + c_{13}N_3); \\ \frac{dN_2}{dt} &= (I(N_2)b_2 - d_2)N_2 - h_2N_2(N_2 + c_{21}N_1 + c_{23}N_3).\end{aligned}$$

In addition, the Allee effect is introduced. According to the Allee effect, when N_3 is extremely low, sexual sp.3 becomes unable to easily find mates and thus the population growth rate is reduced. At the same time, the cost of reproductive interference on self-fertile sp.1 and sp.2 reduces.

The Allee effect, depending on N_3 , is given as follows:

$$A(N_3) = \frac{\alpha N_3}{1 + \alpha N_3},$$

where $N_3 \geq 0$, we have $0 \leq A(N_3) < 1$. Coefficient $\alpha (\geq 0)$ is for the strength of the Allee effect. And therefore,

$$\begin{aligned}I(N_1)A(N_3) &= \frac{N_1}{N_1 + A(N_3)i_{13}N_3}; \quad I(N_2)A(N_3) = \frac{N_2}{N_2 + A(N_3)i_{23}N_3}, \text{ then} \\ \frac{dN_1}{dt} &= (I(N_1)A(N_3)b_1 - d_1)N_1 - h_1N_1(N_1 + c_{12}N_2 + c_{13}N_3); \\ \frac{dN_2}{dt} &= (I(N_2)A(N_3)b_2 - d_2)N_2 - h_2N_2(N_2 + c_{21}N_1 + c_{23}N_3); \\ \frac{dN_3}{dt} &= (A(N_3)b_3 - d_3)N_3 - h_3N_3(N_3 + c_{31}N_1 + c_{32}N_2).\end{aligned}$$

Moreover, the environmental fluctuation f is added. Since f is the effect of decreasing N_{sp} ($sp = 1, 2$ or 3), we can assume that d_{sp} varies randomly (but f is not depending on N_{sp}). Thus,

$$\begin{aligned}\frac{dN_1}{dt} &= (I(N_1)A(N_3)b_1 - d_1(2fR + 1 - f))N_1 - h_1N_1(N_1 + c_{12}N_2 + c_{13}N_3); \\ \frac{dN_2}{dt} &= (I(N_2)A(N_3)b_2 - d_2(2fR + 1 - f))N_2 - h_2N_2(N_2 + c_{21}N_1 + c_{23}N_3); \\ \frac{dN_3}{dt} &= (A(N_3)b_3 - d_3(2fR + 1 - f))N_3 - h_3N_3(N_3 + c_{31}N_1 + c_{32}N_2),\end{aligned}$$

where d_{sp} varies by a random number R ($0 \leq R \leq 1$), and f is the coefficient for the strength of the environmental fluctuation ($0 \leq f \leq 1$); and when $f = 1$ the environmental fluctuation is maximum, that is, $2fR + 1 - f = 2R$ ($0 \leq 2R \leq 2$). The variability over time t has calculated by the difference formula with the discrete degree s ($0 \leq s \leq 1$) as follows:

$$N_{1,t+s} = N_{1,t} + s \frac{dN_{1,t}}{dt}; \quad N_{2,t+s} = N_{2,t} + s \frac{dN_{2,t}}{dt}; \quad N_{3,t+s} = N_{3,t} + s \frac{dN_{3,t}}{dt}.$$

Intense reproductive interference drove the competition to sexual exclusion of self-fertile species. However, even under intense reproductive interference, the Allee effect allowed self-fertile species to survive when the competition started with the lower densities; contrary to the survival of sexual species when the competition started with the higher densities. In such case when environmental fluctuation was enough large to cause extinctions of three species, self-fertile species repeatedly and dominantly occurred. In rice fields, several weeks after rice planting are the period of rice establishment. At the same time, it is usually also a stable time for tadpole shrimps. Therefore, such extreme environmental fluctuations as described above do not need to be taken into account, because the water environment is maintained by rice farmers.

If so, whether an invasive alien *Triops* species will become established in Japanese rice fields or be competitively excluded by the native *Triops* species? It most probably depends on the native *Triops* were present in sufficient numbers to maintain reproducible populations in the rice fields before the invasion of alien species. In other words, it is suggested that we have to keep out any excess application of herbicides, insecticides and other pesticides at the time of rice planting, in order to not eradicate native species. This mathematical competition model shows that if the native species are disappeared, then it would accelerate the growing of alien tadpole shrimps, which could lead to be increasing in pest damages.

6. Ecological and economic impact

6.1 Invasive alien vs. prehistoric naturalised *Triops*

Recently the variation in world tadpole shrimps has been paid much attention, and large number of the synonymies produced by Linder (1952) and Longhurst (1955) have not been substantiated by the later morphological and molecular examinations of many researchers, including me. *Triops granarius* s.l. shows considerable morphological heterogeneity over its formerly stated extensive geographical range (Barnard, 1924; 1929; Gurney, 1924; 1925; Thiéry, 1987; Tiwari, 1951; 1954; Karande and Inamdar, 1959; Rayner and Bowland, 1985; Shanbhag and Inamdar, 1968; Meintjes et al., 1994; Hamer and Rayner, 1995; Naganawa, 2018). Thus, it is impossible by the standards of modern science to regard them as a single species; and there is no taxonomic rationale for it as well.

One thing is certain that the primary mode of reproduction in tadpole shrimps is sexual one. Later, other reproduction modes without males, i.e., hermaphroditism (self-fertilisation) and/or parthenogenesis would have been derived from it. This is because these animals always form haploid germ cells by meiosis in their reproductive organs. Each haploid germ cell must make double the number of chromosomes. And therefore, if a single individual forms two different types in size of germ cells, then it shows self-fertilisation. Otherwise, the germ cells have no difference in size and it means automictic parthenogenesis. In addition, several researchers (e.g., Sassaman et al., 1997; Macdonald III et al., 2011) have proposed the presence of androdioecy in tadpole shrimp reproduction, but more details will be discussed in my another publication.

In the 1910s an American, and in the 1950s a European species of *Triops* also seems to have entered Japan. Originally, Japanese paddy fields had Asian *Triops* species as a prehistoric naturalised animal; this well coincides with the introduction of ancient wet-rice (= Japonica rice) cultivation from mainland China to Japan. A new invasive *Triops* from Western Australia has been reported in the south part of Japan, occasionally forming mixed populations. To date, no case of asexual populations for Asian native species has been reported (nor has evidence of hermaphroditism). This point is very different, i.e., invasive alien species found in Japan, originated from Italy or Serbia, from the USA and from Australia, which are considered sister groups of Asian *Triops*, are all asexual populations without exception.

6.2 Kabuto-ebi Mai — a new challenge

In recent years, the harm of tadpole shrimp has received increasing attention, particularly in southern Australia. Very interestingly, during the course of the present study, I found that the origins of invasive alien tadpole shrimps in Japan are limited to the Japonica rice paddies that have been expanded overseas (Fig. 6). A brand name “Kabuto-ebi Mai” (*Kabuto-ebi* is the Japanese name for tadpole shrimps, *Mai* means rice in Japanese) was popular in Japan for a while. In my opinion, however, rice fields where only invasive alien tadpole shrimps inhabit are never ecologically sound. Although similar attempts have been observed in Yamagata Prefecture etc., Professor Takayuki Nagashima’s group (entomology lab.) at the Tokyo University of Agriculture (Japan) has been working with local laypersons in Chichibu City, Saitama Prefecture, for 20 years to investigate the effectiveness of weed control by tadpole shrimp. Since this animal is extremely sensitive to pesticides, so the rice fields where they live are evidence of pesticide-free cultivation. Will the ‘tadpole shrimp farming by pesticide-free method’ which enables low-cost, labour-saving rice cultivation, spread to other parts of Japan?

The newest species of invasive tadpole shrimps in Japan was brought from Australia to Wakayama Prefecture accidentally by human import about 30 years ago and widely established there (Fig. 7), but no local laypersons, even rice farmers, have been aware of the fact until I began the research (Naganawa, 2018). This proves that the pest damage by invasive alien species to rice crop, at the same time, the efficacy of native species of tadpole shrimps have been underestimated in Japan, respectively. According to the Rice field guide of New South Wales, Australia (Stevens, 2013), (1) Crop damage: tadpole shrimp attack the roots of young seedlings; (2) Management: the impact of tadpole shrimp can be minimised by sowing crops as soon as possible after the fields are flooded. This will ensure the plants are further through the establishment phase by the time tadpole shrimp are large enough to cause plant damage. There are currently no registered pesticides for tadpole shrimp control in Australia. Even so, it would be difficult to apply this to Japanese rice fields as it is. The most important thing is, first of all, to distinguish species of tadpole shrimps; and secondly, to find a way to deal with them that matches their ecological characteristics of the species in question.

Figure 6: Distribution of rice crop areas for three varieties in the world, and estimated intrusion routes of alien (self-fertile) *Triops* species into Japan

Figure 7: Australian tadpole shrimps are in explosive numbers (a rice field in Wakayama Prefecture, Japan)

7. Conclusions

I conducted specimen surveys in 2019 on more than 20 times as many type and reference specimens at several natural history museums in Europe as Longhurst did at that time of his research. As a result, “*Triops granarius*” sensu Longhurst is to be far from a widely-ranging species of tadpole shrimp from Africa to Asia. Hence, I revised it and redescribed herein as 8 independent species in the same genus.

In the wild, multiple closely-related species of tadpole shrimps cannot share the same niche, so there is usually only one constituent species in a single body of water. I, however, found such an interesting case in southern Japan as some rice fields where three species of tadpole shrimps are coexisting. To estimate the pattern of establishment or extinction of alien tadpole shrimps, I developed a simple mathematical competition model based on Tilman’s (1982) that incorporates reproductive interference between two self-fertile species (imported from the USA and Australia) and one related sexual species (i.e., an ancient Asian species of Japan).

My results suggest that if the populations can be managed appropriately, a new method for the selective eradication of harmful invasive tadpole shrimps can be proposed by releasing the native species of tadpole shrimp into the troublesome rice fields. Specifically, by using the competition principle inherent in these animals, without use of any pesticides. **[Note added in proof: Self-fertilisation of an Asian *Triops* species has been confirmed by me for the first time in the world after the present work was accepted for publication.]**

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Appendices

Appendix 1: Synonym lists and taxonomic accounts for the *Triops granarius* s.l. species group

***Triops numidicus* (Grube, 1865), resurrected (Fig. 2 and 3; Appendix 2 and 3)**

Non: *Apus granarius* Lucas, 1864; *Apus numidicus* var. *dybowskii* Braem, 1893; *Apus numidicus* in Uéno (1925);
Apus numidicus var. *sinensis* Uéno, 1925; *Triops granarius* in Longhurst (1955) [in part]; *Apus dispar* in Forró and Brtek (1984);
Triops granarius dispar in Forró and Brtek (1984)

Syn.: *Apus numidicus* Grube, 1865; *Apus dispar* Brauer, 1877; *Apus dukianus* Day, 1880; *Apus numidicus* in Simon (1886) [in part];
Apus numidicus var. *strauchii* Braem, 1893; *Apus somalicus* Wedenissow, 1895; *Apus bottegoi* Del Prato, 1896;
Apus bottegoi in Bouvier (1899); *Apus numidicus* in G. O. Sars (1898); *Apus numidicus* in G. O. Sars (1899);
Apus trachyasps G. O. Sars, 1899; *Apus numidicus* in G. O. Sars (1905); *Apus numidicus* in Stebbing (1910);
Apus trachyasps in Stebbing (1910); *Apus zanoni* Colosi, 1920; *Proterothrips zanoni* in Ghigi (1921);
Proterothrips somalicus in Colosi (1923); *Apus numidicus* in Barnard (1924) [in part]; *Apus numidicus* in Gurney (1924);
Apus numidicus in Pérès (1939); *Triops* cf. *granarius* (sp.3) from Tunisia in Naganawa (2018)

Types.—None designated in the original description (Grube, 1865). Naganawa confirmed that no type of this species is deposited in the Natural History Museum Vienna, Austria; but the type of *Apus dispar* Brauer, 1877 is deposited there. [cf. Type of *Apus trachyasps* G. O. Sars, 1899 is deposited in the South African Museum, Cape Town, South Africa.]

Diagnosis.—Dorsal organ depressed and (sub)triangular or trapezoidal. Carapace oval, its length (including the posterior angles) a little greater than its width, more or less arched, usually convex along whole lateral margin to posterior angle. About 30, varying 25–33 in both sexes, abdominal segments are uncovered by carapace. Number of apodal (= limbless) segments normally 13–14 (males) or 10 (females), varying 12–15 (males) or 9–13 (females). Carina lacks spines. Sulcus cut not very deep, on the concave margin about 20–34 denticles (almost small rounded protuberances) on each side (42–63 in total). Fourth endite of 1st limb usually as long as, or a little longer than carapace. Males having strong scales on furcal filaments (= caudal rami) as Asian *Triops* spp. Furcal filaments in females are so long, but in males about as long as carapace (including posterior angles).

Type locality.—Algeria.

Distribution.—North Africa (Sahara Desert: Algeria, Tunisia, Libya, Sudan), East Africa (Somalia, Kenya), South Africa (Namibia, Botswana, South Africa), Arabia, Afghanistan.

Remarks.—Synonymy complex; even so, in the light of current scientific standards, Barnard's (1929) synonymy is the most reliable one. Since Longhurst's (1955) revision this species has been confused under the name of "*Triops granarius*" for several couple of decades; or more specifically, "*Triops numidicus*" and "*Triops granarius*" have been used for the same populations by different authors. These two are, however, morphologically and molecular genetically independent each other (Naganawa, 2018; Naganawa, personal observation). *Triops numidicus* is the species originated from Africa; whereas *Triops granarius* [emended by me in the text] is the species distributing in East Asia only. The former is by far the commonest and the most widely distributed *Triops* species throughout Africa, and partly reaches over the Arabian Peninsula; but not confirmed in more east than Afghanistan. And therefore, I suggest herein the binomen "*Triops granarius*" be restricted as '*Triops granarius* s.str.' to the population in East Asia. This suggestion is not an option under the International Code of Zoological Nomenclature. Thus, I propose resurrecting the species name '*Triops numidicus*'.

***Triops namaquensis* (Richters, 1886), resurrected (Fig. 2 and 3; Appendix 2 and 3)**

Non: *Triops granarius* in Longhurst (1955) [in part]

Syn.: *Apus namaquensis* Richters, 1886; *Apus namaquensis* G. O. Sars, 1899 [non *Apus namaquensis* Richters, 1886; as a new species];
Apus sculleyi G. O. Sars, 1899; *Apus elongatus* Thiele, 1907 [nom. nov. for *Apus namaquensis* G. O. Sars, 1899];
Apus namaquensis in Stebbing (1910); *Apus sculleyi* in Stebbing (1910); *Apus sculleyi* in Daday de Deés (1913);
Apus namaquensis in Barnard (1924); *Apus namaquensis* in Barnard (1929); *Triops* cf. *granarius* (sp.2) from South Africa in Naganawa (2018)

Types.—None designated in the original description (Richters, 1886). [cf. Types of *Apus namaquensis* G. O. Sars, 1899 and *Apus sculleyi* G. O. Sars, 1899 are deposited in the South African Museum, Cape Town, South Africa.]

Diagnosis.—Dorsal organ depressed and triangular. Carapace round, only very slightly, if at all, longer than width and flattened. Lateral margins usually slightly concave near the posterior angles. About 36 (males) or 30 (females) abdominal segments are uncovered by carapace. Number of apodal segments normally 15 (males) or 13 (females), varying 14–17 (males) or 12–14 (females). Carina lacks spines and smooth, neckline almost distinguishable. Sulcus cut not very deep, on the concave margin about 24 teeth on each side (46–54 in total). Fourth endite of 1st limb usually a little shorter than carapace. Males having strong scales on furcal filaments as Asian *Triops* spp. Furcal filaments in females are usually not longer than median length of carapace, in males often considerably shorter.

Type locality.—Namaqualand, Namibia.

Distribution.—South Africa (Namibia, South Africa), East Africa (Kenya), North Africa (Algeria).

Remarks.—Two male specimens from Algeria, labelled '*Apus Thielei*' [undescribed] by Daday de Deés (Daday Phyllopoda Collection, accession no. NHMUS D1911-37; I/B-41) were examined by me at the Hungarian Natural History Museum, Budapest in 2019. And I confirmed enough key characters among them to be assigned to this taxon. Longhurst (1955) synonymised this species as a junior synonym of his "*Triops granarius*", but this is an independent species morphologically and molecular genetically (Naganawa, 2018; Naganawa, personal observation). Consequently, I propose resurrecting the species name '*Triops namaquensis*'.

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Appendix 1 (continued)

***Triops sudanicus* (Brauer, 1877)**, resurrected (Fig. 2 and 3; Appendix 2 and 3)

Non: *Apus sudanicus* var. *chinensis* Braem, 1893; *Triops granarius* in Longhurst (1955) [in part]

Syn.: *Apus sudanicus* Brauer, 1877; *Apus abyssinicus* Richters, 1886; *Apus sudanicus* var. *braueri* Braem, 1893; *Thriops uebensis* Colosi, 1922; *Apus numidicus* in Barnard (1924) [in part]; *Apus sudanicus* in Barnard (1929); *Triops* cf. *granarius* (sp.1) from Namibia in Naganawa (2018)

Types.—Natural History Museum Vienna, Austria.

Diagnosis.—Dorsal organ conical and oval. Carapace oval, slightly longer than width, more or less arched, lateral margins not concave near posterior angles. 22–23 (males) or 19–21 (females) abdominal segments are uncovered by carapace. Number of apodal segments 9–13 (males) or 7–8 (females). Carina lacks spines. Sulcus cut very deep, on the concave margin 19–25 teeth on each side (38–50 in total). Fourth endite of 1st limb a little longer than carapace (males) or about as long as carapace (females). Males not having strong scales on furcal filaments as Asian *Triops* spp.; rather having regular rows of thorns. Furcal filaments about as long as carapace, including posterior angles.

Type locality.—Tura el Chadra at Bahr el Abiad, Sudan.

Distribution.—North Africa (Sudan, Egypt), South Africa (Namibia, South Africa).

Remarks.—Longhurst (1955) synonymised this species as a junior synonym of his “*Triops granarius*”, but this is an independent species morphologically and molecular genetically (Naganawa, 2018; Naganawa, personal observation). Because of this, I propose resurrecting the species name ‘*Triops sudanicus*’.

***Triops orientalis* (Tiwari, 1951)**, resurrected (Fig. 2 and 3; Appendix 2 and 3)

Syn.: *Apus orientalis* Tiwari, 1951 [non 1952, Longhurst’s mistake]; *Triops* cf. *granarius* (sp.5) from Maharashtra in Naganawa (2018)

Diagnosis is as the same as the original description (Tiwari, 1951).

***Triops mavliensis* (Tiwari, 1951)**, resurrected (Fig. 2 and 3; Appendix 2 and 3)

Syn.: *Apus mavliensis* Tiwari, 1951 [non 1952, Longhurst’s mistake]; *Triops* cf. *granarius* (sp.4) from Udaipur in Naganawa (2018)

Diagnosis is as the same as the emended description by Shanbhag and Inamdar (1968).

***Triops dybowskii* (Braem, 1893)**, resurrected (Fig. 2 and 3; Appendix 2 and 3)

Non: *Apus granarius* Lucas, 1864; *Apus numidicus* Grube, 1865; *Apus granarius* in Simon (1886); *Apus sudanicus* var. *chinensis* Braem, 1893; *Apus granarius* in Gauthier (1934); *Triops granarius* in Longhurst (1955) [in part]

Syn.: *Apus numidicus* in Simon (1886) [in part]; *Apus numidicus* var. *dybowskii* Braem, 1893; *Apus granarius* in G. O. Sars (1899); *Apus granarius* in G. O. Sars (1901); *Apus asiaticus* Gurney, 1921 [nom. nov. for *Apus granarius* in G. O. Sars (1901)]; *Apus asiaticus* in Gurney (1924); *Apus numidicus* in Uéno (1925, 1927); *Apus numidicus* in Uéno (1935) [in part]; *Apus numidicus* in Koba (1938b); *Apus granarius* in Uéno (1940); *Apus dispar* in Forró and Brtek (1984); *Triops granarius dispar* in Forró and Brtek (1984); *Triops* sp. Mandalgobi in Naganawa (2018); in Sun and Cheng (2019); *Triops* sp. Choir in Naganawa (2018); in Sun and Cheng (2019); *Triops* sp. Baikol Olkhon Is. in Naganawa (2018); in Sun and Cheng (2019); *Triops granarius* from Mongolia in Naganawa (2018); in Sun and Cheng (2019); *Triops granarius* from South Ural in Naganawa (2018); in Sun and Cheng (2019); *Triops granarius* from Inner Mongolia in Sun and Cheng (2019)

Types.—None designated in the original description (Braem, 1893). According to the Museum of Natural History, University of Wrocław (Poland), all of the deposited Braem’s specimens were lost during war time. And therefore, designation of **neotypes** is desirable: specimens from the type locality (Daday Phyllopoda Collection, accession no. **NHMUS D1911-20; I/B-23**) are selected herein (see also Appendix 1).

Diagnosis.—Dorsal organ depressed and triangular. Carapace oval, its length (including the posterior angles) a little greater than its width, more or less arched, usually convex along whole lateral margin to posterior angle. About 30 (males) or 27 (females) abdominal segments are uncovered by carapace. Number of apodal segments 12–14 (males) or 9–11 (females). Carina lacks spines or has very small teeth in the middle of the neckline. Sulcus cut not very deep, on the concave margin about 30 teeth on each side (54–70 in total). Fourth endite of 1st limb much shorter than carapace. Males having strong scales on furcal filaments. The length of furcal filaments in females are so long, but in males shorter than the length of uncovered abdominal segments.

Type locality.—Darasum Village, Karymsky District, Zabaikalsky Krai (Dauria [= Transbaikalia] Region), Siberia, Russia.

Distribution.—Siberia (Ural region, Lake Baikal catchment, Transbaikalia region), Mongolia, China (Inner Mongolia and Peking), Pakistan.

Remarks.—I confirmed that this species was misidentified as ‘*Apus dispar*’ by Daday de Deés; however, *Apus dispar* is a junior synonym of *Triops numidicus*. In addition, this species has been confused under the name of “*Triops granarius*” by different authors since G. O. Sars’ (1899) report. For example, Uéno incorrectly described this species as *Apus numidicus* in several papers published before 1940; and later changed the name to “*Triops granarius*” (Uéno, 1940). In fact, these species are independent morphologically and molecular genetically each other (Naganawa, 2018; Naganawa, personal observation); and enough key characters are present in the morphology of the teeth on the concave margin of the carapace to differentiate. Thus, I propose assigning the new name ‘*Triops dybowskii*’ to this species, in order to distinguish from *Triops numidicus* and *Triops granarius*. *Apus numidicus* var. *dybowskii* Braem, 1893 was described by Fritz Braem, German zoologist, worked at the University of Breslau (currently Wrocław, Poland). I confirmed that the specimens were lost during the Battle of Wrocław on January of 1945 (World War II) [source: Jolanta Jurkowska; curator of Invertebrates Collection in the Museum of Natural History, University of Wrocław, Poland].

Appendix 2: Specimens used as the basis for descriptions in this study; see also Forró and Brtek (1984) for further information

***Triops numidicus* (Grube, 1865)**

Adult ♂♂, total length (from anterior margin to hind end of telson: TL) on average 21.51 mm (range 16.62–24.48 mm; $n = 6$); carapace length (from anterior margin to hind end of median carina: CL) 10.17 mm (9.87–10.89 mm), adult ♀♀ [all egg sacs are empty], TL 19.47 mm (17.84–21.10 mm; $n = 2$); CL 10.59 mm (10.14–11.04 mm), juvenile ♀, TL 20.21 mm; CL 10.99 mm, deposited in the Department of Zoology, the Hungarian Natural History Museum (NHMUS); Daday Phyllopoda Collection (shown as ‘Collectio Dadayana Phyllopoda’ on the label), accession no. NHMUS D1911-24; I/B-27 [9 individuals in 1 tube]. Locality: Tilremt, Région des Dayas, Algeria [Note: without data on sampling details, probably collected in early 1900s; collector: unknown]

Adult ♂♂, TL 26.72 mm (24.47–28.96 mm; $n = 16$); CL 9.81 mm (9.70–9.92 mm), ovigerous ♀, TL 21.88 mm; CL 10.22 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-25; I/B-28 [17 individuals in 1 tube]. Locality: Tilremt, Région des Dayas, Algeria [Note: without data on sampling details, probably collected in early 1900s; collector: unknown]

Adult ♂♂, TL [unavailable by poor condition] ($n = 3$); CL [also unavailable], ovigerous ♀, TL 26.00 mm; CL 14.76 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-26; I/B-29 [4 individuals in 1 tube]. Locality: Ouled Sidi Brahim, M’Sila Province, Algeria [Note: without data on sampling details, probably collected in early 1900s; collector: unknown]

Adult ♂♂, TL 12.33 mm (10.16–14.13 mm, $n = 9$); CL 5.72 mm (5.09–6.22 mm), adult ♀♀ [all egg sacs are empty], TL 10.15 mm (9.06–11.89 mm, $n = 6$); CL 4.62 mm (4.35–5.52 mm), deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-34; I/B-38 [15 individuals in 1 tube]. Locality: Pfanne bei Sekuma, Kalahari Desert, Botswana [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; labelled ‘*Apus Schultzei*’ (undescribed) by Daday de Deés]

Juveniles ($n = 17$), deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-38; I/B-42 [17 individuals in 1 tube]. Locality: Kinangop, Kenya [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; identified and labelled ‘*Apus trachyaspis*’ by Daday de Deés]

***Triops namaquensis* (Richters, 1886)**

Adult ♂♂, TL 30.37 mm (28.46–32.27 mm, $n = 2$); CL 14.30 mm (14.19–14.41 mm), deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-37; I/B-41 [2 individuals in 1 tube]. Locality: Ouargla and Timassinin, Sahara Desert, Algeria [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; labelled ‘*Apus Thielei*’ (undescribed) by Daday de Deés]

***Triops sudanicus* (Brauer, 1877)**

Adult ♂♂, TL 35.07 mm (31.85–38.29 mm; $n = 2$); CL 18.24 mm (17.83–18.65 mm), adult ♀ [both egg sacs are empty], TL 24.80 mm; CL 13.37 mm, juveniles, TL 20.46 mm (20.04–21.13 mm; $n = 3$); CL 12.37 mm (11.94–12.72 mm), deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-36; I/B-40 [6 individuals in 1 tube]. Locality: Tura el Chadra, Bahr el Abiad, Sudan [Note: same as the type locality; without data on sampling details, probably collected in early 1900s; collector: unknown]

***Triops dybowskii* (Braem, 1893)**

Neotypes: adult ♂, TL 32.47 mm; CL 12.25 mm, ovigerous ♀, TL 27.13 mm; CL 12.29 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. **NHMUS D1911-20; I/B-23** [2 individuals in 1 tube]. Locality: Darasum Village, Karymsky District, Zabaikalsky Krai (Dauria [= Transbaikalia] Region), Siberia, Russia [Note: same as the type locality; without data on sampling details, probably collected in early 1900s; collector: unknown; misidentified and labelled ‘*Apus dispar*’ by Daday de Deés]

Adult ♂, TL [unavailable by poor condition]; CL 17.11 mm, ovigerous ♀, TL 33.54 mm; CL 16.39 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-21; I/B-24 [2 individuals in 1 tube]. Locality: Peking, China [Note: indicated as ‘♂’ on the original label written by Daday de Deés, but one of two specimens is undoubtedly female; without data on sampling details, probably collected in early 1900s; collector: unknown; misidentified and labelled ‘*Apus dispar*’ by Daday de Deés]

Adult ♂♂, TL 35.95 mm (32.40–39.49 mm; $n = 2$); CL 15.28 mm (15.00–15.55 mm), ovigerous ♀, TL 27.27 mm; CL 15.01 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-22; I/B-25 [3 individuals in 1 tube]. Locality: Borobalgasun, Ordos, Inner Mongolia, China [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; misidentified and labelled ‘*Apus dispar*’ by Daday de Deés]

Adult ♂, TL 28.70 mm; CL 15.63 mm, ovigerous ♀, TL 29.76 mm; CL 18.16 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-19; I/B-22 [2 individuals in 1 tube]. Locality: Tsitsik-nor (Tsetseg Nuur [= Tsetseg Lake]) catchment, Tsetseg sum., Khovd aimag, Mongolia [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; misidentified and labelled ‘*Apus dispar*’ by Daday de Deés]

Adult ♂, TL 34.79 mm; CL 13.19 mm, ovigerous ♀, TL 33.30 mm; CL 15.13 mm, deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-23; I/B-26 [2 individuals in 1 tube]. Locality: Liari, Lasbela District, Balochistan Province, Pakistan [Note: without data on sampling details, probably collected in early 1900s; collector: unknown; misidentified and labelled ‘*Apus dispar*’ by Daday de Deés]

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Appendix 2 (continued)

***Triops granarius* s.str. (Lucas, 1864)**

Adult ♂♂, TL 32.56 mm (30.02–35.99 mm; $n = 3$); CL 19.44 mm (18.49–20.73 mm), ovigerous ♀♀, TL 34.15 mm (32.73–36.42 mm; $n = 3$); CL 21.99 mm (20.44–23.69 mm), deposited in the NHMUS; Daday Phyllopoda Collection, accession no. NHMUS D1911-35; I/B-39 [6 individuals in 1 tube]. Locality: Manchuria, China [Note: same as the type locality; without data on sampling details, probably collected in early 1900s; collector: unknown; labelled '*Apus Mackenseni*' (undescribed) by Daday de Deés]

Adult ♂, TL 38.5 mm; CL 21.0 mm, ovigerous ♀, TL 32.0 mm; CL 22.5 mm [after Uéno's (1925) original description; currently both specimens are severely damaged and cannot be measured], deposited in the Kyoto University Museum (KUM); Center for Ecological Research Collection, accession no. KUM CER Ar.085. Locality: Jukkenbou, the former Mukden (now Shenyang, Liaoning Province), China [Note: apart from the original specimen bottle, there is an outer storage bottle that labelled as 'Type' for *Apus numidicus* var. *sinensis* Uéno, 1925; however, when and by whom the label was attached are unknown]

Appendix 3: Key to the *Triops granarius* s.l. species group (African-Indian, Siberian-Asian)

African *Triops granarius* s.l. species*

1. Dorsal organ depressed, triangular. Furcal filaments ♂ with strong scales as Siberian-Asian *Triops* spp.
 - 1-1. Carapace oval
 - 1-1-1. Apodal segments ♂ 12–15, ♀ 9–13 (on average ♂ 13–14, ♀ 10) ... NUMIDICUS
 - 1-1-2. Apodal segments ♂ 10, ♀ unknown ... DADAYI
 - 1-2. Carapace round. Apodal segments ♂ 14–17, ♀ 12–14 (on average ♂ 15, ♀ 13) ... NAMAQUENSIS
2. Dorsal organ conical, oval. Furcal filaments ♂ with regular rows of thorns only.
 - Carapace oval. Apodal segments ♂ 9–13, ♀ 7–8 ... SUDANICUS

Indian *Triops granarius* s.l. species*

1. Dorsal organ depressed, triangular. Furcal filaments ♂ with strong scales as Siberia-Asian *Triops* spp. Carapace oval
 - 1-1. Apodal segments ♂ 11–13, ♀ 10–12 (on average ♂ 12, ♀ 11) ... ORIENTALIS
 - 1-2. Apodal segments ♂ 10–12, ♀ 7–9 (on average ♂ 11, ♀ 8) ... MAVLIENSIS

Siberian-Asian *Triops granarius* s.l. species*

1. Dorsal organ depressed, triangular. Furcal filaments ♂ with strong scales
 - 1-1. Carapace oval. Apodal segments ♂ 12–14, ♀ 9–11 ... DYBOWSKII
 - 1-2. Carapace ♂ round, ♀ elongated (Buddhist temple bell-shaped). Apodal segments ♂ 8–10, ♀ 4–7 ... GRANARIUS

*African and Indian species groups have been confirmed by molecular phylogenetic analysis (Naganawa, 2018) to be genetically in the same clade, but independent zoogeographically; African-Indian and Siberian-Asian species groups to be independent both genetically and zoogeographically. The numbers of apodal (= limbless) segments of each as a criterion for *Triops* species-level classification were determined comprehensively by taking into account actual measurements under a binocular stereomicroscope by me, as well as retrieved data from the original described literature of all available synonyms.