

## Relationship between morphological and genetic trees of marine chironomid species

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**Abstract** Relationships between morphological and genetic trees of 4 marine orthoclad and related genera, *Clunio*, *Semiocladius*, *Telmatogeton* and *Thalassosmittia*, of the dipteran family Chironomidae as well as a terrestrial genus *Smittia* and a freshwater genus *Cricotopus* were examined. In the genetic tree, there were 3 lineages; *Smittia*, *Semiocladius* and other 4 genera, comprising *Cricotopus*. *Telmatogeton* and *Thalassosmittia* formed an intimate clade in spite of their different affiliations to the subfamily. *Semiocladius* and *Clunio* made a small clade in the morphological tree, whereas *Telmatogeton* and *Thalassosmittia* did not form a clade by themselves. *Cricotopus* participated in the largest cluster. These results suggest that *Clunio*, *Telmatogeton* and *Thalassosmittia* have derived from a freshwater ancestor and there be some morphological parallelisms.

**Key words:** adult male, genetic tree, marine chironomid, Orthoclaadiinae, morphological tree

### INTRODUCTION

Chironomidae comprises about 10000 species in the world (Armitage et al., 1995) and is most widely distributed among the aquatic insects (Pinder, 1986). Physiological and ecological studies of some marine species (*Clunio*, *Telmatogeton* and *Pontomyia*) were reported (Hashimoto, 1975, 1976; Sunose et al., 1982). However, there have been practically no studies on morphological as well as genetic relationships between marine orthoclad species.

In this paper, the species of the subfamily Orthoclaadiinae and a related subfamily were studied on morphological and genetic aspects. Three genera of marine Orthoclaadiinae: *Clunio*, *Semiocladius* and *Thalassosmittia* and a marine genus, *Telmatogeton* of Telmatogetoninae as well as *Cricotopus*, a freshwater genus and *Smittia*, a terrestrial genus, all widely distributed in Japan, were selected. Morphological tree was constructed by examination of some characters of males, genetic one was constructed on the basis of the sequence of *COI* region of mtDNA, and then both trees were compared from the evolutionary view.

### MATERIALS AND METHODS

#### 1. Sampling

Adult male samples were collected mainly by sweeping of swarms or by sucking on the rocks and buildings on the shore during Jun. '04 to Oct. '06. in the south-western Japan, including Hiroshima,

Kochi, Kagoshima and Okinawa Prefectures. Wings were removed and mounted dry on slide glass, the body was macerated in hot 10% KOH solution, washed in water, and mounted in gum-chloral solution. Identification was performed using keys provided by Sasa et Kikuchi (1995) and Tokunaga (1937).

## 2. Morphological analysis

Nineteen characters (Chara. Nos. 34-52 in Cranston, 2000) were determined for states and a most parsimonious tree was constructed by PAUP4.0b (Swofford, 2000).

## 3. DNA preparation

Template DNA was prepared from the samples using DNeasy Tissue Kit (Quiagen, Tokyo, Japan), according to the manufacturer's instruction.

## 4. PCR

A part (105bp) of *COI* region of mtDNA was amplified by PCR. PCR was performed with a mixture of a template DNA (50ng), primers (Simon et al., 1994: 1718, 5'-GGAGGATTTGGAAATTGAGTTCC-3'; Ohsugi, 2005: YU1718R, 5'-GTAGGTACGGCAATAATTAT-3') in a thermal cycler (Mastercycler personal, Eppendorf, Hamburg, Germany) in the following protocol: preheating at 94°C for 10min. → (denaturation at 94°C for 30sec. → annealing at 40°C for 30sec → extension at 72°C for 2min) × 45cycles → extension at 72°C for 7min.

## 5. Sequencing

Sequencing was performed with a genetic analyzer 3130xl (Applied Biosystem, USA). Multiple alignment was performed with a Clustal W soft (Thompson et al., 1994).

## 6. Dendrogram

Genetic distance between the individuals was calculated with Kimura-2-parameter and dendrogram was constructed by neighbor-joining method using PHILIP 3.6 (Felsenstein, 1995).

# RESULTS

## 1. Morphological data

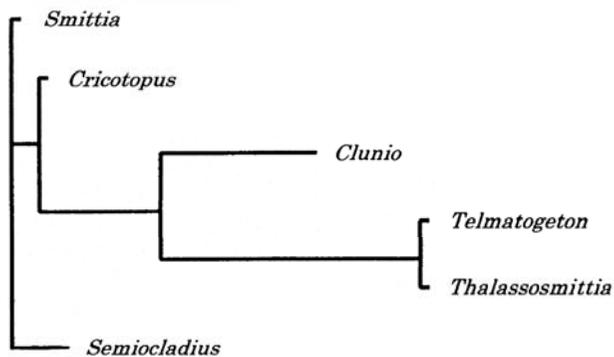
A data matrix for interpretation of phylogeny of 6 genera: 19 characters were scored in Table 1.

Table 1. Data matrix for interpretation of phylogeny of 6 genera of Orthocladiinae and a relative. Nineteen characters were scored.

<i>Chunio tsushimensis</i>	1001002100021010000
<i>Cricotopus sylvestris</i>	1200001020001000000
<i>Semiocladus endocladiae</i>	1002002011011010000
<i>Smittia aterrima</i>	1100001000001100000
<i>Telmatogeton japonicus</i>	0002110121021000300
<i>Thalassosmittia nemalionis</i>	1000000001021000000

## 2. Genetic relationship (Fig. 1)

There were 3 lineages; a terrestrial genus *Smittia*, a marine genus *Semiocladius* and other 4 genera. Thus, a freshwater genus *Cricotopus* participated in a cluster constructed by 3 marine genera. *Telmatogeton*, a marine genus of Telmatogetoninae, and *Thalassosmittia*, a marine genus of Orthoclaadiinae, formed a small cluster.



### 0.1 substitution

Fig. 1. Genetic trees of 4 marine genera, *Clunio*, *Semiocladius*, *Telmatogeton* and *Thalassosmittia*, as well as terrestrial *Smittia* and freshwater *Cricotopus* as outgroups.

## 3. Morphological relationship (Fig. 2)

*Semiocladius* and *Clunio* made a small cluster. *Telmatogeton* and *Thalassosmittia* did not form a cluster by themselves. *Smittia* participated in a large cluster of 4 marine genera. *Cricotopus* participated in a large cluster at the last.

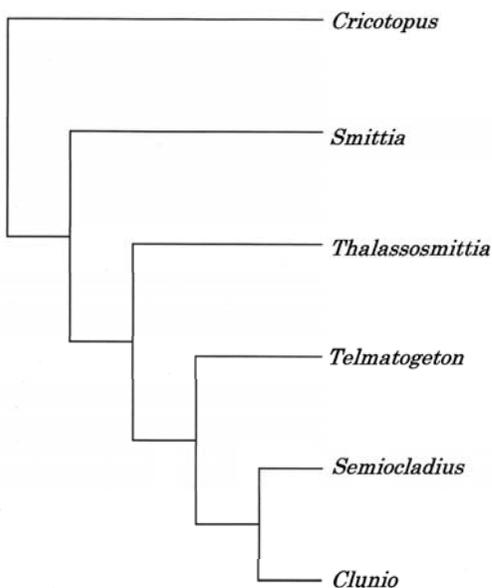


Fig. 2. Morphological trees of 4 marine genera as well as a terrestrial and a freshwater genus as outgroups.

## DISCUSSION

Among 6 genera studied, *Smittia* mainly comprises terrestrial species (Kobayashi, 1993). *Cricotopus* comprises many freshwater species and a few of blackish water species. Indeed, *C. sylvestris*, *C. trifasciatus* and *C. tricinctus* were often observed in estuaries (Kawai et al., 2000). On the other hand, *Clunio*, *Semiocladius*, *Telmatogeton* and *Thalassosmittia* contains only marine species.

In a morphological tree, *Smittia* affiliated to a clade of marine 4 genera, and *Cricotopus* constructed the largest cluster. On the other hand, in the genetic tree, *Clunio*, *Telmatogeton* and *Thalassosmittia* constructed a clade with *Cricotopus*. Thus, these 3 marine genera might have derived from a freshwater ancestor. *Clunio* might have invaded into marine environment at the first, and *Telmatogeton* and *Thalassosmittia* at the second. *Semiocladius* might have invaded into marine environment still earlier than other 3 marine genera in spite of its low level of morphological adaptation to sea shore life. On the other hand, *Smittia* and *Semiocladius* are common in having a subapical strong straight seta on the antenna (Wiederholm, 1989), although these are distant both in genetic and morphological trees. Further, *Semiocladius* and *Clunio* made a small cluster in the morphological tree whereas they are rather distantly related in the genetic tree. These results suggest that *Clunio*, *Telmatogeton* and *Thalassosmittia* have derived from a freshwater ancestor at the different periods and that there be some morphological parallelisms. Besides, *Telmatogeton* and *Thalassosmittia* made a small cluster in spite of each affiliation to different subfamilies, suggesting an invalidity of the subfamily Telmatogetoninae..

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## 海産ユスリカ種の形態学的系統樹と遺伝学的系統樹の関係

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**要旨** 海産4属, *Clunio*, *Semiocladius*, *Telmatogeton* および *Thalassosmittia* と陸生の *Smittia*, 淡水性の *Cricotopus* 属のエリユスリカ亜科と近縁亜科の形態学的系統樹と遺伝学的系統樹の関係を調べた。遺伝学的系統樹では3系統, すなわち *Smittia*, *Semiocladius* と *Clunio* を含む他の4属に分かれた。さらに, *Telmatogeton* と *Thalassosmittia* は別亜科にも拘わらず小さなクラスターを形成した。形態学的系統樹では *Semiocladius* と *Clunio* は小さなクラスターを形成したが, *Telmatogeton* と *Thalassosmittia* はこれらだけではクラスターを形成しなかった。*Cricotopus* は最後に大きなクラスターに加わった。これらの結果は, *Clunio*, *Telmatogeton* と *Thalassosmittia* は淡水種の祖先から派生したこと, そして形態学的形質の中にいくらかの平行進化が存在することを示唆する。

**キーワード**: 海産ユスリカ, エリユスリカ亜科, 雄成虫, 遺伝子系統樹, 形態系統樹