

## Diploid-triploid Hybrid Complex of the Spined Loach *Cobitis sinensis* and *C. longicarpus*(Pices, Cobitidae)

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The cobitid hybrid complex of *C. sinensis* and *C. longicarpus* occurs commonly in the upper streams of the Nakdong River, Korea where it is sympatric with the bisexual species, *C. sinensis* and *C. longicarpus*. The specimens of hybrid complex which are all females accounted for approximately 40% of all cobitid fish observed. These cobitid complex having the unique cloudy specks on body sides appeared to be morphologically intermediate between *C. sinensis* and *C. longicarpus*. The chromosomes of hybrid complex were composed of both diploid form of 49 and triploid form of 73. This forms are tentatively presumed to be a unisexual complex originating from hybrid between *C. sinensis* and *C. longicarpus*.

### Introduction

The genus *Cobitis* is represented by 9 species or subspecies in Korea, of which 5 species are endemic to Korea. The characters of their body color patterns and secondary sexual characters at the base of pectoral fin in male are taxonomically useful in distinguishing the species of the genus *Cobitis*(Kim and Son, 1984; Kim and Lee, 1987). In the Nakdong river drainage, *C. sinensis* and *C. longicarpus* are commonly and have similar ranges at some localities.

Among the cobitid specimens collected in the Nakdong River system, many individuals showed intermediate forms such as hybrid between *C. sinensis* and *C. longicarpus*. In the course of the study on these putative hybrid specimens, we found that they are almost females, outnumbering the parent species at some localities, and occurring both diploids and triploids. So we report here the diploid and triploid unisexual forms originating from hybrid between *C. sinensis* and *C. longicarpus* based on the morphological and karyological data.

### Materials and methods

All specimens were collected from the upper streams of the Nakdong river at the Nanwon-gun, Chollabuk-do, during March 1988 to July 1989. Specimens are deposited at the Department of Biology, Chonbuk National University(CUB). Morphological analyses were made mainly following those of Hubbs and Lagler(1964) and a hybrid index was calculated according to

Hubbs *et al*(1943). Chromosome preparations were obtained from the flame drying method of gill epithelium and kidney tissues of the samples, using after colchicine and hypotonic treatments. Slides were made on metaphase spreads from each fish. The chromosome identification was designated according to Levan *et al*.(1964). In nomination of putative hybrid complex between *C. sinensis* and *C. longicorpus*, we temporally designated as *Cobitis sinensis-longicorpus* according to Schultz(1969).

## Results

**Occurrence of hybrid complex and sex ratio :** In the present study, 495 fish specimens of the genus *Cobitis* were collected and analyzed. Of these specimens 125 individuals (25.3%) are *C. sinensis* and 157 individuals (31.7%) are *C. longicorpus*, while 231 individuals (43.0%) are the putative hybrid complex population as shown in Table 1. The average adult sex ratio were slightly in favor of females in both *C. sinensis* and *C. longicorpus*, while *C. sinensis-longicorpus* individuals are all female except 2 males of 212.

Table 1. Sex related occurrence frequencies in the species and hybrid complex of the genus *Cobitis* collected from the water area of Inwol-myon, Nam-won-gun, Chollabuk-do, Korea in 1988-1989

Collection date	<i>C. sinensis</i>		<i>C. longicorpus</i>		<i>C. sinensis-longicorpus</i>	
	male	female	male	female	male	female
Mar. 1, 1988	2	5	5	8	—	13
Apr. 5, 1988	—	2	6	9	—	3
May. 6, 1988	—	2	—	3	—	4
Jun. 7, 1988	6	6	7	8	1	50
Jun. 20, 1988	5	5	7	8	—	12
Jul. 28, 1988	4	6	8	10	—	6
Aug. 5, 1988	2	4	3	3	—	20
Nov. 23, 1988	2	3	3	4	—	20
Feb. 22, 1989	10	11	2	13	—	26
Apr. 4, 1989	13	21	2	2	—	31
May. 7, 1989	1	2	6	7	—	4
Jul. 5, 1989	7	6	7	26	1	22
Total number (%)	125 (25.3%)		157 (31.7%)		213 (43.0%)	
Percent females	58.4		64.3		99.1	

**Morphological characters :** Living and preserved specimens of the Cobitid fish species usually are identified by the character of color patters on the body sides. *C. sinensis* has a series of round brownish blotches on the middle part of the body sides, while *C. longicorpus* has a series of brownish crossbands and a black spot at the first crossband. However it is remarkable that the band patterns of *C. sinensis-longicorpus* are intermediate between two species and has the peculiar cloudy color patterns on the upper part of the body sides (Fig.1). Table 2 summerizes

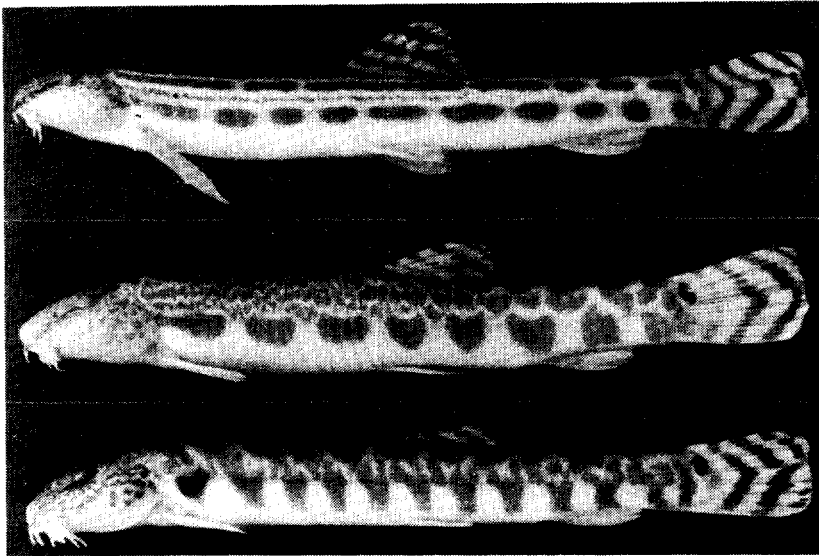


Fig. 1. *Cobitis sinensis*, CUB 15643, 76.9mm SL (top); *Cobitis sinensis-longicorpus*, CUB 15644, 95.0mm SL (middle); *Cobitis longicorpus*, CUB 15645, 74.4mm SL (bottom).

Table 2. Comparison of morphometric characteristics of the *Cobitis sinensis*, *C. longicorpus* and their hybrid complex collected from Namwon-gun, Chollabuk-do, Korea in 1988. A thirty specimens measured on each species and their their hybrid complex. Mean±SD

	<i>C. sinensis</i>	<i>C. sinensis-longicorpus</i>	<i>C. longicorpus</i>	Hybrid index
Standard length, mm	61.3-92.9	54.0-112.3	64.1-122.5	
In % of standard length				
predorsal length	51.0±1.3 (48.6-54.6)	53.3±1.4 (51.2-55.8)	55.3±1.6 (53.6-58.3)	0.53
preventral length	53.5±1.3 (52.4-54.6)	54.7±1.7 (51.9-57.3)	56.6±1.6 (53.6-60.8)	0.38
preanal length	79.9±2.2 (77.4-82.5)	80.9±1.9 (78.1-83.9)	81.4±1.5 (78.4-84.4)	0.67
body depth	13.6±1.1 (12.5-15.3)	12.8±5.9 (10.1-18.3)	13.7±1.3 (11.2-15.1)	*
head length	20.9±0.6 (19.6-22.0)	21.0±0.7 (19.6-22.5)	21.1±0.8 (19.8-23.2)	0.50
pectoral-ventral distance	32.7±1.6 (29.9-35.9)	34.7±2.1 (31.1-37.9)	36.3±2.2 (33.5-42.9)	0.55
caudal peduncle depth	8.2±0.5 (7.3-9.5)	9.4±0.9 (7.9-12.0)	9.6±0.8 (8.5-11.4)	0.86
In % of head length				
eye diameter	17.9±1.4 (14.9-20.3)	16.5±0.9 (14.8-18.0)	14.7±1.2 (11.9-16.3)	0.44
interorbital width	16.3±0.9 (15.1-21.2)	20.6±2.5 (18.1-26.4)	25.5±2.4 (22.6-30.4)	0.47
3rd barbel length	17.9±2.0 (15.1-21.2)	19.7±2.7 (17.1-24.4)	21.5±1.7 (18.0-28.1)	0.50
In % of caudal peduncle length				
caudal peduncle depth	61.2±8.8 (46.5-92.8)	65.7±7.6 (46.5-84.5)	74.1±8.6 (63.0-100.0)	0.33

\* Hybrid value less than the mean for either parent.

for 11 morphometric characters. Hybrid indices are intermediate for seven characters, of predorsal length, preventral length, preanal length, head length, pectoral-ventral distance, interorbital width and the third barbel length. And they are closer to *C. longicarpus* in two characters and closer to *C. sinensis* in two characters. In situations like this, almost mean value in characters of hybrid complex investigated were within the range for both parents, however the mean value of body depth in hybrid complex was lower than the mean for either parent.

**Chromosome counts:** Chromosome counts from gill and kidney tissue of *C. sinensis*, *C. longicarpus* and *C. sinensis-longicarpus* showed in table 3. The modal chromosome number of specimens examined was 48 in *C. sinensis* and 50 in *C. longicarpus*, however *C. sinensis-longicarpus* showed diploid-triploid forms(Fig. 2). Chromosome numbers of diploid 49, and triploid had 73 respectively(Fig. 3). And among 176 *C. sinensis-longicarpus* individuals investigated, the diploid form is 36 individuals (20%) and the triploid form is 140 individuals (80%). The diploid karyotype of *C. sinensis-longicarpus* in the present study consists of 19 metacentrics and submetacentrics and 30 subtelocentrics and acrocentric chromosomes and was consisted of 28 metacentrics-submetacentrics and 45 subtelocentrics-acrocentric chromosomes (Fig. 3).

Table 3. Karyotype formular of *Cobitis sinensis*, *C. longicarpus* and their hybrid complex

Biotype	chromosome No.	karyotypic formular*	FN**
<i>Cobitis sinensis</i>	48	18msm-30stt	66
<i>C. longicarpus</i>	50	20msm-30stt	70
<i>C. sinensis-longicarpus</i> (diploid form)	49	19msm-30stt	68
<i>C. sinensis-longicarpus</i> (triploid form)	73	28msm-45stt	101

\* meta submeta-subtelo telo centric chromosome

\*\* fundamental number

## Discussions

The presence of hybrids in population is usually inferred when sympatric specimens are found that are morphologically intermediate between two species (Hubbs, 1955; Greenfield *et al.*, 1972; Das and Nelson, 1988). Based on the results of morphological analysis on the genus *Cobitis* populations in the present study, almost characters of the putative hybrid *C. sinensis-longicarpus* specimens are intermediate form between *C. sinensis* and *C. longicarpus*. For the presence of the intermediate form like this, we can consider only three possible explanations: 1) it is a component of one of two bisexual species, 2) it is a natural hybrid of on going hybridization between *C. sinensis* and *C. longicarpus*, and 3) it is separate lineage. In this case *C. sinensis* is distinguished from *C. longicarpus* by the number of diploid chromosomes and their color patterns; the former for 48, the latter for 50. However all specimens of *C. sinensis-longicarpus* with a peculiar color pattern above the body side. The diploid chromosomes with

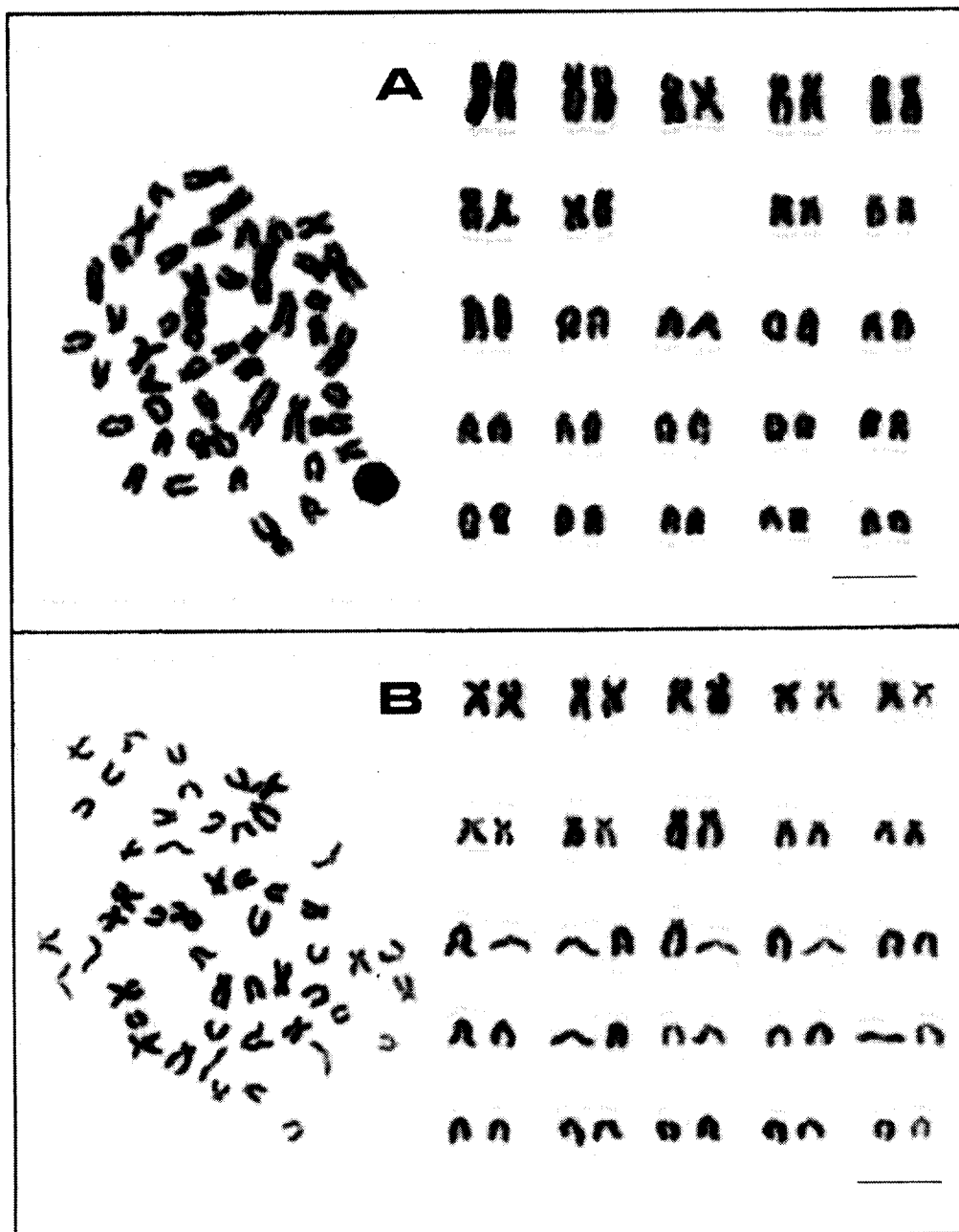


Fig. 2. Metaphase plates and karyotypes of *Cobitis sinensis*(A; 2n=48) and *Cobitis longicarpus*(B; 2n=50). Bars indicate 5μm.

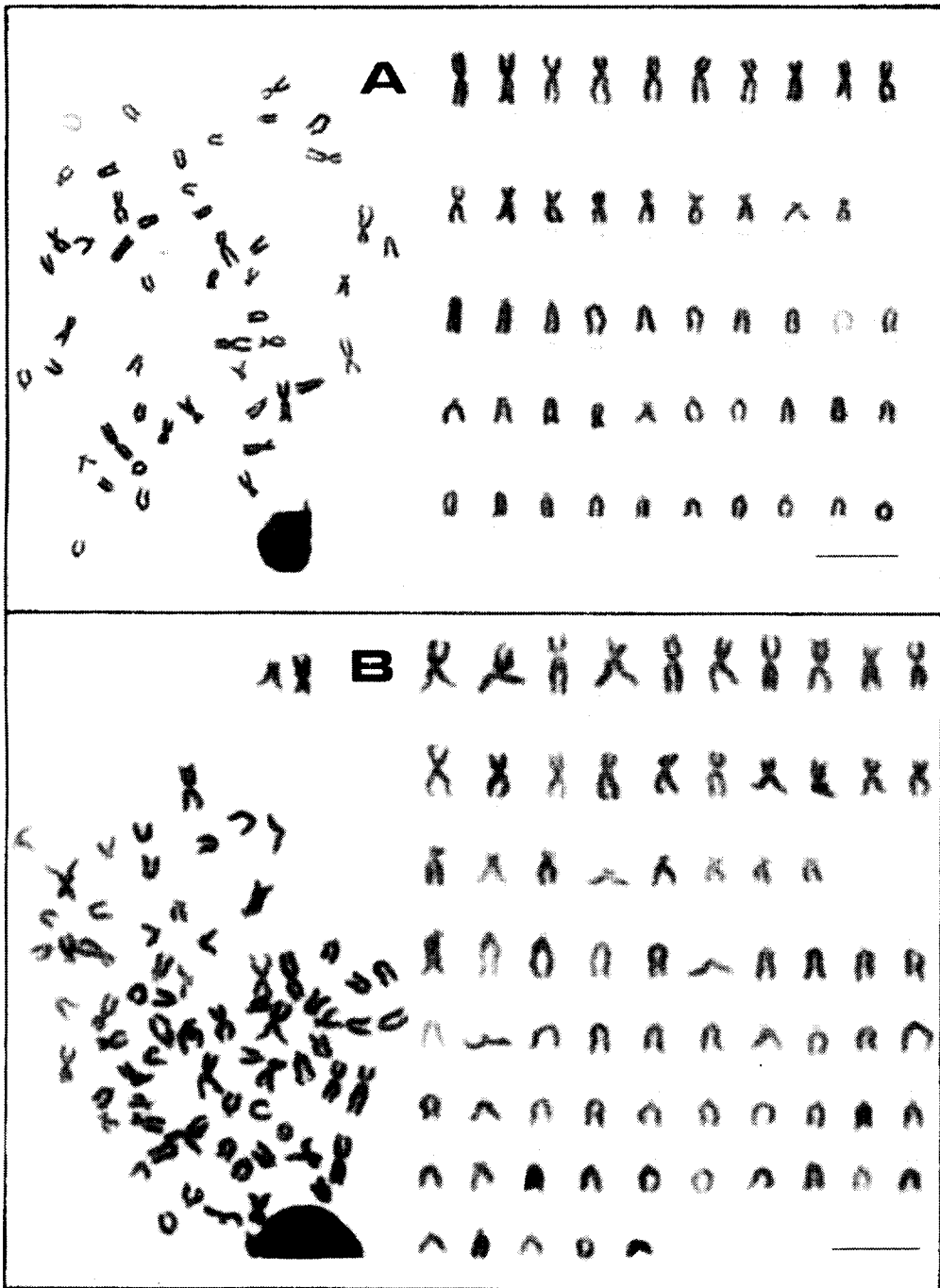


Fig. 3. Metaphase plates and karyotypes of the diploid form (A;  $2n=49$ ) and the triploid form (B;  $3n=73$ ) of *Cobitis sinensis-longicorpus*. Bars indicate  $5\mu\text{m}$ .

49 of hybrid individuals are intermediate between that of *C. sinensis* and *C. longicorpus*. By the way the high frequency of all female allotriploids probably ensures that they are not natural hybrids of on going hybridization between two species. In this case, the triploid with 73 chromosomes would include displaced sets from *C. sinensis* and haploid set from *C. longicorpus*, the former stemming from an aborted meiotic division such as other triploid unisexual fishes (Schultz, 1977; Dawley and Goddard, 1988; Vrijenhoek *et al.*, 1989). The amazon molly, *Poecilia formosa*, a gynogenetic fish was first known unisexual vertebrate (Hubbs and Hubbs, 1932). The discoverers of its unisexuality recognized early that it was probably of hybrid origin, as it was almost exact morphological intermediate between the two parent species. Vasil'ev *et al.* (1989) reported the triploid-tetraploid gynogenetic forms of the genus *Cobitis* including two bisexual species, *C. taenia* and *C. granoei* in Moscow river drainage. In conclusion the morphological and karyological features on the hybrid complex demonstrate that *C. sinensis*-*longicorpus* complex are not simple natural hybridization between the two species, but it is tentatively presumed to be a unisexual lineage originating from hybrid between the *C. sinensis* and *C. longicorpus*. Further works might reveal more about the taxonomic position and reproductive mechanisms of the present cobitid complex.

## References

- Das, M. K. and J. S. Nelson. 1988. Hybridization between northern redbelly dace (*Phoxinus eos*) and finescale dace (*Phoxinus neogaeus*) (Osteichthyes: Cyprinidae) in Alberta. *Can. J. Zool.*, 67: 579-584.
- Dawley, R. M. and K. A. Goddard. 1988. Diploid-triploid mosaics among unisexual hybrid of the minnow *Phoxinus eos* and *Phoxinus neogaeus*. *Evolution* 42(4): 649-659.
- Greenfield, D. W., and T. Greenfield. 1972. Introgressive hybridization between *Gila orcuti* and *Hesperoleucas symmetricus* (Pisces: Cyprinidae) in the Cuyama River Basin, California. I. Meristic, morphometric and breeding. *Copeia*, 1972: 549-589.
- Hubbs, C. L. 1955. Hybridization between fish species in nature. *Syst. Zool.*, 4: 1-20.
- Hubbs, C. L. and L. C. Hubbs. 1932. Apparent parthenogenesis in nature, in the form of a fish of hybrid origin. *Science* 76: 628-630.
- Hubbs, C. L., L. C. Hubbs, and R. E. Johnson. 1943. Hybridization in nature between species of catostomid fishes. *Contrib. Lab. Vert. Biol. Univ. Michigan*, No. 27, 76pp.
- Hubbs, C. L. and K. F. Lagler. 1964. *Fishes of the great Lakes region*. The Univ. Michigan Press. 19-26.
- Kim, I. S. and Y. M. Son. 1984. *Cobitis choii* a new cobitid fish from Korea. *Kor. J. Zool.*, 27(1): 49-55.
- Kim, I. S. and W. O. Lee. 1987. A new subspecies of cobitid fish (Pisces: Cobitidae) from the Paikchon Stream, Chollabuk-do, Korea. *Kor. J. Syst. Zool.*, 3(1): 57-62.
- Levan, A., K. Fredga, and A. A. Sandbag. 1964. Nomenclature for centromeric position on chromosome. *Hereditas*, 52: 201-220.
- Schultz, R. J. 1969. Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Amer. Nat.*, 103: 605-619.
- Schultz, R. J. 1977. Evolution and ecology of unisexual fishes. *Evol. Biol.*, 10: 277-331.
- Vasilev, V. P., K. D. Vasileva and A. G. Osinov. 1989. Evolution of dipliod-triploid-tetraploid complex

in fishes of the genus *Cobitis*(Pisces, Cobitidae). pp.156-169. In: Evolution and ecology of unisexual vertebrates, edited by R. M. Dawley and J. P. Bogart, Bull., 466, New York State Museum, Albany, New York.

Vrijenhoek, R. C., R. M. Dawley, C. J. Cole, and J. P. Bogart. 1989. A list of the known unisexual vertebrates. pp. 19-23. In: Evolution and ecology of unisexual vertebrates, edited by R. M. Dawley and J. P. Bogart, Bull., 466, New York State Museum, Albany, New York.

## 기름종개와 왕종개의 잡종복합군의 2배체와 3배체에 관하여

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기름종개 *C. sinensis*와 왕종개 *C. longicarpus*가 함께 서식하는 낙동강상류의 여러 수역에서 이들 사이의 잡종이라고 추측되는 개체들이 높은 빈도 (43%)로 출현하였는데 그들은 모두 암컷이었다. 이들 잡종 개체들은 체측에 특이한 구름무늬 반문을 가지면서 형태적으로는 기름종개와 왕종개의 중간형질을 보여주고 그들의 염색체수는 2배체형인 49개이거나 3배체형인 73개이었다. 이들은 기름종개와 왕종개 사이에 잡종에서 유래된 후 이 종과는 독립된 단성복합집단(unisexual complex) 이라고 생각한다.