

Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of new species

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Abstract Many rotifers are considered to be cosmopolitan species including *Epiphanes senta*, which has been reported from all continents including Antarctica. To determine the potential for cryptic speciation in this species, we examined morphology, mating behavior and genetic divergence in three populations: (1) a tropical alpine lake on Mauna Kea (HI, USA), (2) temporary rock pools of the Chihuahuan Desert (TX, USA), and (3) floodplain habitats of the Oder River (Germany). Morphological differences among these populations are restricted to the surface structure of diapausing eggs and the shape of the rami. In crossmating experiments, German males never fertilized females from the Texan population. Texan males mated with hatching females from the German population. However, juvenile mortality among these fertilized females reached 40% and was significantly higher than juvenile mortality after intrapopulation mating (2%). Surviving females did

not produce viable diapausing eggs. Hawaiian males readily mated with newborn Texan females, which produced viable offspring. They also copulated with females from the German population, but these females never produced diapausing eggs. Genetic distance among the three populations was considerable, ranging from 10.9 to 12.5% for 648 bp of the mitochondrial *cox1* gene. Morphological, reproductive, and genetic differentiation among the studied populations demonstrates *E. senta* is actually a species complex. Three new species within the *E. senta* species complex are described.

Keywords Cryptic speciation · Species complex · Rotifers · *Epiphanes* · New species

Introduction

Historically, many rotifer species have been considered to have worldwide distributions due to their high dispersal capabilities (Ruttner-Kolisko, 1974; Koste, 1978; Wallace & Snell, 2001), but recently it has been shown that so-called cosmopolitan species may in fact be cryptic species complexes (e.g. Ciroso-Pérez et al., 2001a; Gómez et al., 2002; Derry et al., 2003; Gilbert & Walsh, 2005; Gómez, 2005). Cryptic speciation has been studied more extensively in other freshwater microinvertebrates such as Copepoda and Cladocera (e.g. Taylor & Hebert, 1992; Lee, 2000; Lee & Frost, 2002; Dodson et al., 2003; Penton et al.,

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2004; Kim et al., 2006) and many marine invertebrates (Knowlton, 2000). Genetic differentiation may be considerable due to reproductive isolation and/or geographic barriers to gene flow. Typically divergences of >5% are used to delineate species based on sequence data (Avisé & Walker, 1999; Hebert et al., 2003). Previous molecular studies have shown high levels of sequence divergence among cosmopolitan rotifer species (*Brachionus calyciflorus* (Gilbert & Walsh, 2005); *Brachionus plicatilis* (Gómez et al., 2002); assorted species (Derry et al., 2003)). Reproductive isolation and substantial genetic differentiation has taken place in geographically distant populations of *B. calyciflorus* (Gilbert & Walsh, 2005) and sympatric populations of *B. plicatilis* (Gómez 2005).

Having been reported from all continents (De Ridder & Segers, 1997), including Antarctica (José de Paggi & Koste, 1984), *Epiphanes senta* (Müller, 1773) is generally considered to be cosmopolitan (Koste, 1978; Jersabek, 2003). However, documented distribution patterns are disjunct, with active populations of often occurring in eutrophic waters only in the spring or summer months, followed by long periods of the year when they are absent (Wesenberg-Lund, 1930; Ruttner-Kolisko, 1974). With this sort of temporal distribution pattern, researchers may have overlooked populations. To determine the extent of divergence within *E. senta*, we sampled three distant populations (Germany, Texas, Hawaii) and determined morphological variation, reproductive isolation and genetic distance of the mtDNA *cox1* gene.

Material and methods

Sampling

Individuals were collected from three populations of *Epiphanes senta* in Germany, in the northern Chihuahuan Desert of Texas (USA), and on the main island of Hawaii (USA). The German sample was obtained from temporarily flooded areas of the Oder River floodplain (53°02.071' N, 14°15.626' E) near the city of Schwedt (Brandenburg) as well as in a nearby small permanent pond (52°56.331' N, 14°01.324' E). Individuals from Texas were taken from two temporary rock pools (31°55.485' N, 106°02.538' W and 31°55.492' N, 106°02.544' W)

at Hueco Tanks State Historic Site (El Paso Co.). The Hawaii population was found in Lake Waiau (19°48.84' N, 155°28.86' W, 3,968 m), a tropical alpine lake on Mauna Kea. All samples were collected with a 30- μ m plankton net. Clonal lineages were started from single females in Petri dishes (90 mm diameter) filled with modified MBL medium (Stemberger, 1981) and fed with *Cryptomonas erosa* var. *reflexa*. These cultures were used to obtain females, males, and eggs for further investigation.

Rotifers and diapausing eggs were observed and measured using a Zeiss Axioskop and a Leica DMRB with Syncroscope[®] AutoMontage System at 100 \times and 400 \times magnification. Trophi were prepared using a NaClO solution (Clorox[®] bleach) and examined under the light microscope at 400 \times and 1,000 \times magnification and by SEM (Hitachi S-4800). Measurements of trophi were made from digital images taken with a SPOT INSIGHT camera (Diagnostic Instruments, Inc.) mounted on the Zeiss Axioskop and using the SPOT image analysis software, version 4.6 (Diagnostic Instruments, Inc.). The measurements were made on 10 individuals taken from 10 distinct clonal lineages for each of the German and Texan population and on 10 individuals from a field sample of the Hawaiian population. Measurements were analyzed statistically using the JMP statistical software package (SAS Institute, 2001).

Crossbreeding experiments

Clones from each geographical area were used for crossbreeding experiments. Clones were derived from single amictic females isolated from field collections. Because of difficulties in maintaining many clonal cultures simultaneously, clones were inbred and the resulting diapausing eggs were stored in darkness at 20–25°C until needed for crossing experiments. At this time, single diapausing eggs were hatched to establish new clonal lineages.

Reciprocal crosses within and among geographical regions were conducted. However, experiments with Hawaiian females and males from the other populations were not conducted, because mictic females seldom appeared in these cultures. The few males that were produced were used in mating experiments with females from the German and Texan populations.

Individual females of each clone were isolated from mass cultures and reared in 2 ml of culture medium (MBL medium and *Cryptomonas erosa* var. *reflexa*) in 24-well tissue culture plates at 15°C. This volume was used to induce the production of mictic offspring. Culture medium was exchanged daily. Eggs deposited by each amictic female were collected daily as were males that hatched from mictic eggs. Female eggs with fully developed embryos produced by one clone were individually transferred to 96-well tissue culture plates into 50 µl of culture medium together with 4 males (<24 h old) of another clone. The next day, after females had hatched, another 200 µl of culture medium were added. The following day, males were removed. Females were cultured until they produced eggs. Production of diapausing eggs (successful mating) and male eggs (unsuccessful mating) by mictic females was recorded. Females that died before they reproduced were recorded to document juvenile mortality. Details of all mating experiments are given in Table 1. Diapausing eggs from each cross were collected and kept at 20–25°C for 3 weeks and then at 12°C in plastic Petri dishes (diameter 50 mm) filled with fresh medium. This procedure has been shown to induce hatching (unpublished observations). The diapausing eggs were checked for hatching and the medium was exchanged every 4 days. Differences in juvenile mortality were analyzed using the G-test of the statistical software package JMP (SAS Institute, 2001).

DNA sequencing

DNA was isolated and amplified from fresh or ethanol preserved animals. The Hawaiian template

was obtained from a mass culture initiated from a field collection that was maintained for >20 generations. Templates for the German and Texas strains were derived from a clone from each site. Total numbers of individuals per extraction was approximately 50. Several templates were prepared for each lineage. Primers used to amplify the mitochondrial *cox1* gene are LCO1490: 5'-GGTCAACAAATCA-TAAAGATATTGG-3' and HCO2198: 5'-TAAACTT CAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994). Amplification products were examined by electrophoresis to verify their size and cleaned with GeneClean kits (BioRad) before sequencing. Products were sequenced directly using SequiTherm kits (EpiCentre Tech.) and run on a LI-COR 4200 Series automated sequencer. All genes were sequenced at least twice in both directions. Sequences were aligned using Clustal W (Thompson et al., 1994) and then adjusted manually. An additional *cox1* sequence for a Mexican *E. senta* isolate was obtained through GeneBank (Accession #72153636) and added to the distance analysis. Uncorrected genetic distance (“p”) calculations adjusted for missing data were done using PAUP4.0b10 (Swofford, 2002) using UPGMA.

Results

Morphological differentiation

Females and males of the three populations are morphologically very similar and are represented by the general morphology in Fig. 1. Morphological differentiation among populations is limited to the trophi (Fig. 2) and to the surface structure and form of

Table 1 Details of crossbreeding experiments

Experiment	Number of female eggs used	Number of hatching amictic females	Number of hatching mictic females	Juvenile mortality (%)
German males—German females	47	15	31	2.1
Texan males—Texan females	185	56	113	8.6
Texan males—German females	82	33	16	40.2
German males—Texan females	36	0	36	0
Hawaiian males—German females	18	4	14	0
Hawaiian males—Texan females	8	2	6	0

The second column gives the total number of subitaneous eggs with female embryos used in each experiment. Third and fourth columns give the number of the hatching females that could be typed as amictic and mictic, respectively, after they reproduced. The fifth column gives the percentage of hatching females which died before they reproduced and therefore could not be typed

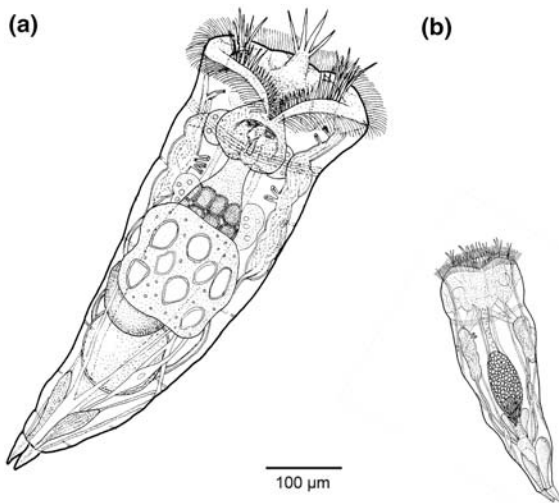


Fig. 1 *Epiphanes senta* species complex, habitus. (a) Female, ventral; (b) male, ventral. Drawings were made from observations under the light microscope using individuals of *E. ukera*

diapausing eggs (Fig. 3). The trophi differ in the shape of their rami in dorsal view (Fig. 2). The basal windows of the rami are much smaller in individuals from the Hawaiian population than in individuals from the other two populations. In the Hawaiian population the basal window extends over $7.2 \pm 0.5\%$ of the ramus area (mean ± 1 s.e., $n = 10$; 95% Confidence Interval (CI) 6.2–8.3%). In the German and the Texan populations, the basal window covers $13.8 \pm 1.1\%$ and $14.4 \pm 0.4\%$, respectively (mean ± 1 s.e., $n = 10$). The 95% CIs are 11.3–16.3% for the German population and 13.4–15.4% for the Texan population. These differences among the populations are highly significant (ANOVA; $F_{2,27} = 28.7$, $P < 0.0001$). Pairwise comparisons show that the Hawaiian population differs significantly from the two other populations in this character (Tukey–Kramer honest significant difference tests, $P = 0.001$). In the German and Hawaiian populations the basal windows are semicircular to oval in shape, whereas in the Texan population they are tear-shaped. The length/width ratio of the basal window in the Texan population is 3.0 ± 0.1 (mean ± 1 s.e., $n = 10$; 95% CI 2.7–3.3). In the German and the Hawaiian populations the length/width ratios of the basal window are 2.5 ± 0.4 and 2.5 ± 0.1 , respectively (95% CIs: German population 2.2–2.8, Hawaiian population 2.2–2.7). These differences are statistically significant (ANOVA; $F_{2,27} = 5.7$, $P = 0.009$).

Pairwise comparisons show that the Texan population differs significantly from the other two populations in this character (Tukey–Kramer honest significant difference tests, $P = 0.05$). The number of unci teeth was found to be variable within populations: females having either 4 or 5 large unci teeth and 3–6 small unci teeth.

Diapausing eggs produced in Texan and Hawaiian populations are ovoid and characterized by a smooth surface while those of the German population are almost spherical and the egg surface is covered by an irregular pattern of ridges (Fig. 3).

Crossbreeding experiments

Results of the crossbreeding experiments are summarized in Table 2. Intrapopulation crosses with clones from the Texan and the German population yielded viable diapausing eggs. Fertilization rates were 84.1% ($n = 113$ mictic females) and 100% ($n = 31$ mictic females) for crosses within Texan and German populations, respectively. Diapausing eggs produced in intrapopulation crosses could be induced to hatch, hatching rates were 25.3% ($n = 95$) for the Texan population and 75% ($n = 24$) for the German population. In mass cultures of Hawaiian clones females almost exclusively reproduced aamictically. Mixis only occurred in a few instances. In these cases, the production of diapausing eggs in the cultures also was observed. Mating between males from the German population and females from the Texan population did not yield any diapausing eggs. None of the mictic females was fertilized and all produced male offspring ($n = 36$). In the crossbreeding experiment with Texan males and German females, males readily copulated with mictic as well as aamictic females. However, the females in this experiment experienced very high mortality during the juvenile period. 40.2% of all females died before they could deposit an egg ($n = 82$). Significantly fewer females (2.1%), died during the juvenile period when German females were fertilized by males of their own clone ($n = 47$; G -test; $P < 0.0001$, $\chi^2 = 28.59$). However, 25% of mictic females that reached maturity ($n = 16$) started to produce diapausing eggs, but failed to deposit viable eggs. Eggs produced by these females were empty or filled with deteriorated yolk, or eggs with an incomplete shell

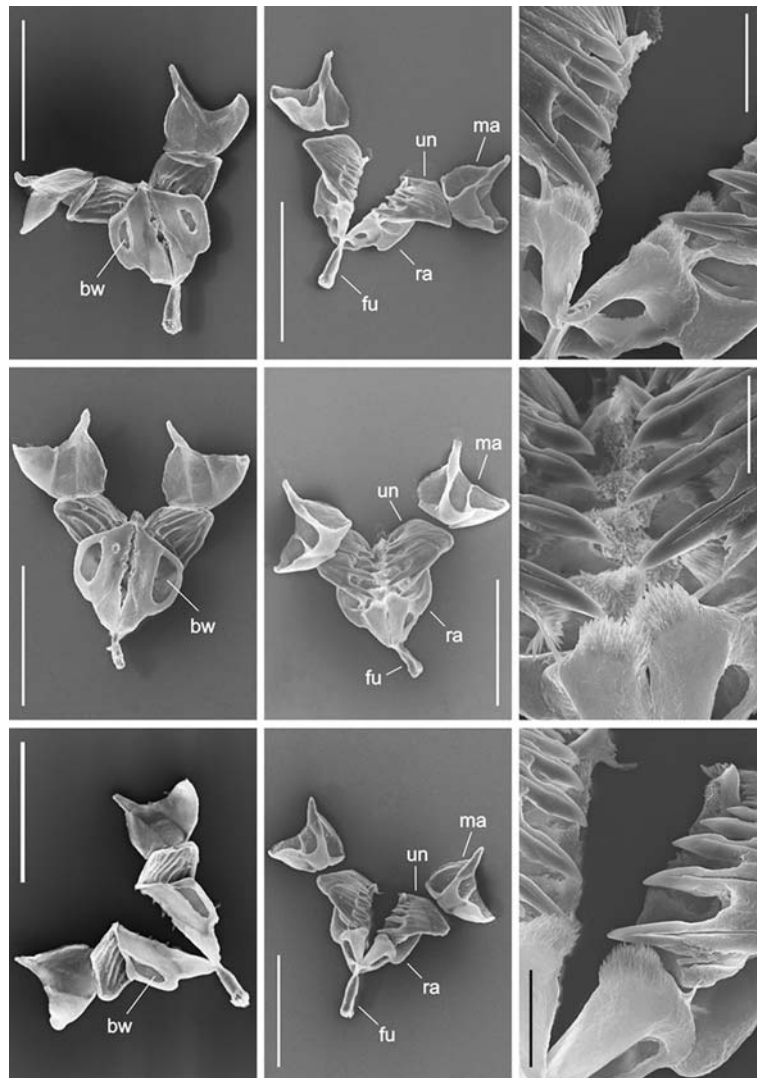


Fig. 2 *Epiphanes senta* species complex, SEM photographs of the trophi. *Top row*: Hawaiian population (*E. hawaiiensis*); *center row*: German population (*E. ukera*); *bottom row*: Texan population (*E. chihuahuaensis*). *Left column*: dorsal view;

center column: ventral view; *right column*: detail of the inner margins of the rami. Scale bars: left and center column 50 μm ; right column 10 μm . Abbreviations: fu = fulcrum, ra = ramus, un = uncus, ma = manubrium, bw = basal window

were deposited. The remaining mictic females produced male offspring. In the crossbreeding experiments with Hawaiian males copulated with females both German and Texas populations. Whereas none of the German mictic females was fertilized and all produced male offspring ($n = 14$), Texan mictic females were fertilized by Hawaiian males and produced diapausing eggs (66%, $n = 6$). Hatching occurred in six out of 21 diapausing eggs that these fertilized females produced.

Genetic variation in mtDNA sequences

A total of 648 bp of the *cox1* gene were analyzed from each population. Sequence divergence was high with a minimum of 10.9% (Table 3). Of the three populations that we studied, the Texan population displayed the highest divergence with 12.2% and 12.5% from the other populations. The Mexican strain showed most sequence similarity to the Hawaiian population (4.6% divergence).

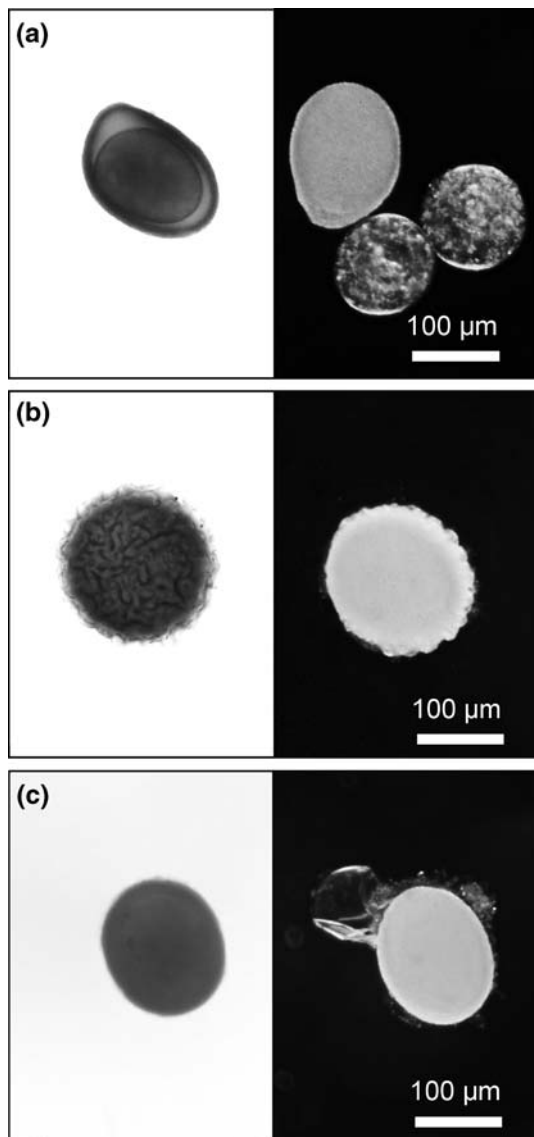


Fig. 3 Light microscopy images of diapausing eggs from the three studied populations of the *Epiphanes senta* species complex. (a) Hawaiian population (*E. hawaiiensis*). *Left*: brightfield illumination; *right*: darkfield illumination (to the right of the diapausing egg are two subitaneous eggs). (b) German population (*E. ukera*). *Left*: brightfield illumination (compound image with Synchronoscopy[®] AutoMontage System); *right*: darkfield illumination. (c) Texan population (*E. chihuahuaensis*). *Left*: diapausing egg in brightfield illumination (Synchronoscopy[®] AutoMontage); *right*: diapausing egg in darkfield illumination. Attached to the egg is an empty subitaneous eggshell and some detritus

Discussion

Our results demonstrate that *Epiphanes senta* is not a cosmopolitan species but comprises a species

complex. The high genetic divergence found in the mitochondrial DNA sequence shows that gene flow between the Hawaiian population and the Texan as well as the German population has not occurred for long periods of time. The amount of divergence in the *cox1* sequence falls within the range of divergences between most of the congeneric species pairs from a broad selection of invertebrate phyla analyzed by Hebert et al. (2003). It is also in a similar range as that found by Gómez et al. (2002) in the *Brachionus plicatilis* species complex. Sequence divergence between the Texan *Epiphanes* population and the other two populations is almost as high as between-lineage divergence in the *B. plicatilis* complex which ranged from 12 to 23% (Gómez et al., 2002). It is difficult to estimate the divergence time of the Texan population from the other two populations from our mtDNA data since making inferences from divergence rates in other invertebrate taxa is problematic (Thomas et al., 2006). However, given a sequence divergence of about 10–12% among our populations, it is likely that each has been on a distinct evolutionary path driven by local environmental conditions.

In addition, reproductive isolation exists between the German population and Texan and Hawaiian populations, respectively. Even though Texan males copulated with German females, only unviable diapausing eggs were produced indicating a possible postzygotic isolation mechanism. Also higher female mortality after mating, compared to mortality after intrapopulation mating, suggests that some kind of incompatibility exists between sperm or other compounds of the semen of Texan males and the physiology of the German females. German males display mate guarding behavior towards females of their own population (Schröder, 2003) but do not respond to females of the Texan or the Hawaiian population (unpublished data). No reproductive isolation was found between Texan and Hawaiian populations. However, these results may not be conclusive because isolation mechanisms that may exist in nature often break down under artificial experimental conditions (Meier & Willmann, 2000). It seems reasonable to assume that reproductive isolation exists between Hawaiian and Texan populations under natural conditions, since mixis was rarely observed in cultures of the Hawaiian *Epiphanes* whereas it occurred regularly in cultures of Texan clones. This may indicate that mixis is induced by

Table 2 Results of crossbreeding experiments with clones from the different populations

		Female		
		Oder floodplains (Germany)	Chihuahuan Desert (Texas)	Lake Waiiau (Hawaii)
Male	Oder floodplains (Germany)	Diapausing eggs	Male offspring only	?
	Chihuahuan Desert (Texas)	High mortality, unviable eggs	Diapausing eggs	?
	Lake Waiiau (Hawaii)	Male offspring only	Diapausing eggs	Diapausing eggs (produced in cultures)

See text for details

Table 3 Genetic distance among populations (uncorrected p values) of the *Epiphanes senta* complex for the *cox1* sequences

	German population	Hawaiian population	Texan population	Mexican population	<i>Epiphanes brachionus</i>
German population	–	0.109	0.125	0.138	0.174
Hawaiian population		–	0.122	0.046	0.169
Texan population			–	0.137	0.190
Mexican population				–	0.166

Distances to *Epiphanes brachionus* from El Paso, Co. TX are given for comparison

different cues and sexual reproduction would not occur in both populations under the same conditions. Further, the possibility of interbreeding does not negate the existence of either parent species (Wheeler & Platnick, 2000b).

Although individuals from the three populations are morphologically very similar, differences exist in trophi morphology as well as in the shell structure of the diapausing eggs. Moreover, the morphology of diapausing eggs found in the three populations is in each case different from that previously described in the literature. Ehrenberg (1838), Plate (1885) and de Beauchamp (1965) studied populations of *E. senta* from sites in Berlin and Brandenburg (Germany), Bonn (Germany), and near Paris (France), respectively. Ehrenberg (1838) shows a drawing of a diapausing egg with a shell covered with tiny spines. However, he claims that these spines are bacteria of the genus *Hygrocrocis*. Plate (1885) and de Beauchamp (1965) both described diapausing eggs where spines are part of the egg shell structure. It seems likely that Ehrenberg mistook the spines for bacterial cells. The description of spiny diapausing eggs by these workers in Germany and France (Ehrenberg, 1838; Plate, 1885; de Beauchamp, 1965) suggests the potential of additional species within the *E. senta* complex, possibly in sympatry with the German species described in this study since it also was found in Brandenburg.

Thus genetic, reproductive, and morphological traits of these three populations support the hypothesis that the three populations represent distinct lineages which have been long separated and can be considered separate species that can be diagnosed by apomorphic morphological characters (Wheeler & Platnick, 2000a, b; Mishler & Theriot, 2000). The Mexican strain is genetically most similar to the Hawaiian population, inferred from the *cox1* gene sequence. However, because only sequence data are available for the Mexican strain it is premature to group it with the Hawaiian population or designate it as a separate species.

The recognition of cryptic species within widely distributed complexes is necessary to accurately interpret ecological interactions among species in their communities. Even in cases where reproductive isolation is incomplete, the potential for ecological differentiation exists. For instance, Weider (1993) found significant effects of species and food level in a number of life history traits in laboratory clones of a hybrid *Daphnia* complex. Ortells et al. (2003) have demonstrated that five members of the *B. plicatilis* species complex occurring in sympatry have unique ecological roles within their habitat and that different patterns of seasonal succession among species helps to explain their coexistence. In laboratory experiments, Ciro-Pérez et al. (2001b) showed that food and

disturbance regimes altered the outcome of competition among three sympatric sibling species. These studies highlight the need for resolution of cryptic species to fully understand local ecological patterns and processes. Finally, on a global scale, accurate species lists are imperative for the management and protection of biodiversity (Padial & De La Riva, 2006).

Description of *Epiphanes ukera* n.sp.

Type locality

Between submerged grasses of inundated meadows in the floodplain of the Lower Oder Valley near Schwedt in Brandenburg, Germany (53°02.071' N, 14°15.626' E). Collected 28 March 2001.

Material examined

The holotype (ANSP 1958) and three paratypes (ANSP 1959, ANSP 1960, and ANSP 1961) are deposited in the rotifer collection of the Academy of Natural Sciences in Philadelphia. Three paratypes are also deposited in the collection of the Laboratory for Environmental Biology at the University of Texas at El Paso (UTEP 4, UTEP5, and UTEP 6).

Differential diagnosis

Epiphanes ukera belongs to the *Epiphanes senta* species complex named after *E. senta* (Müller, 1773). Female and male share the general morphology of the species complex shown in Fig. 1. *Epiphanes ukera* differs from *E. senta* in the shell structure of the diapausing egg. The diapausing egg shell of *Epiphanes senta* sensu stricto is covered with fine spines as described by Plate (1885) and depicted by de Beauchamp (1965) [herewith designated to be the diapausing egg of *Epiphanes senta* (Müller, 1773) as the original description does not include it]. The diapausing egg of *E. ukera* is sometimes oval, but mostly spherical in shape and the shell surface is covered by a pattern of irregular ridges (Fig. 3b).

Description

Female: body illoricate, fusiform, gradually tapering into foot. Two conical toes with large spindle shaped foot glands. Corona with a tuft and two semicircles of cirri. Eyespot not visible. Trophi malleate. Fulcrum short, 1/2 of ramus length. Rami with large basal windows on their dorsal sides. Shape of basal windows oval (Fig. 2). Unci with 4–5 large teeth and 3–4 small teeth.

Male: body fusiform, tapering into foot. Digestive tract reduced. Males display mate guarding behavior and attend female eggs until they hatch (Schröder, 2003).

Measurements

Length of the female 666–774 μm . Length of the male 340–376 μm . Size of male subitaneous egg 104/116–124/128 μm (width/length). Size of female subitaneous egg 140/160–174/190 μm (width/length). Size of diapausing egg 160/170–180/200 μm (width/length). Fulcrum length 24–27 μm . Ramus length 47–54 μm . Dorsoventral projection of ramus 670–960 μm^2 . Size of basal window 60–140 μm^2 . Length of basal window 15–20 μm , width of basal window 6–9 μm .

Distribution and ecology

The species occurs in submerged vegetation of the inundated Oder floodplain and between macrophytes in small permanent ponds in late winter and spring. The water temperature ranged between 4 and 12°C.

Etymology

The species name refers to the region where the type locality is situated, the Uckermark. It is derived from “terra ukera” which is of Slavic origin and means borderland.

Comments

This species was referred to as *E. senta* in previous publications (Schröder, 2001, 2003; Gilbert & Schröder, 2003; Schröder & Gilbert, 2004; Schröder, 2005).

Description of *Epiphanes chihuahuaensis* n.sp.*Type locality*

A temporary rock pool (hueco) at Hueco Tanks State Historic Site, Texas, USA (31°55.492' N, 106°02.544' W). Collected 22 November 2003.

Material examined

The holotype (ANSP 1962) and three paratypes (ANSP 1963, ANSP 1964, and ANSP 1965) are deposited in the rotifer collection of the Academy of Natural Sciences in Philadelphia. Three paratypes are also deposited in the collection of the Laboratory for Environmental Biology at the University of Texas at El Paso (UTEP 7, UTEP 8, and UTEP 9).

Differential diagnosis

Epiphanes chihuahuaensis also belongs the *E. senta* species complex. The diapausing egg of this species differs from those of *E. senta* and *E. ukera* in that its surface is smooth without any spines or ridges. The rami of *E. chihuahuaensis* differ from those of *E. ukera* and *E. hawaiiensis* in shape and size of the basal windows which are tear-shaped and intermediate in size (Fig. 2).

Description

Female: body illoricate, fusiform, gradually tapering into foot. Two conical toes with large spindle shaped foot glands. Corona with a tuft and two semicircles of cirri. Eyespot not visible. Trophi malleate. Fulcrum short, 1/2 of ramus length. Unci with 4–5 large teeth and 3–4 small teeth.

Male: body fusiform, tapering into foot. Digestive tract reduced. As in *E. ukera* males attend female eggs and mate with hatching females.

Measurements

Length of the female 630–738 μm . Length of the male 320–380 μm . Size of male subitaneous egg 94/96–118/

120 μm (width/length). Size of female subitaneous egg 116/130–124/142 μm (width/length). Size of diapausing egg 120/158–140/188 μm (width/length). Fulcrum length 22–29 μm . Ramus length 42–45 μm . Dorsoventral projection of ramus 470–570 μm^2 . Size of basal window 60–100 μm^2 . Length of basal window 15–22 μm , width of basal window 5–6 μm .

Distribution and ecology

The species is found in shallow huecos (0.3–4 m diameter), pools and other temporary habitats. These habitats are filled by rainfall and are dry again after several days to weeks. *E. chihuahuaensis* may appear in the pools a few days after a rainfall event. Rainfall in the Chihuahuan Desert is very unpredictable and mainly occurs from July to September and to a lesser extent during the winter months.

Etymology

The species name refers to the type locality in the northeastern part of the Chihuahuan Desert in North America.

Description of *Epiphanes hawaiiensis* n.sp.*Type locality*

The tropical alpine Lake Waiau (19°48.84' N, 155°28.86' W, elevation 3,968 m) below the summit of Mauna Kea on the main island of Hawaii. Collected 24 August 2004.

Material examined

Holotype (ANSP 1966) and three paratypes (ANSP 1967, ANSP 1968, and ANSP 1969) are deposited in the rotifer collection of the Academy of Natural Sciences in Philadelphia. Three paratypes are also deposited in the collections of the Laboratory for Environmental Biology at the University of Texas at El Paso (UTEP 10, UTEP 11, and UTEP 12) and the Bishop Museum in Honolulu, Hawaii.

Differential diagnosis

The species is distinguished from *E. ukera* and *E. chihuahuaensis* by the shape of the rami in dorsal view, which have much smaller semicircular basal windows (Fig. 2). The basal window extends only over 7% of the dorsal side of the ramus, whereas in *E. ukera* and *E. chihuahuaensis* it covers about 14% of the dorsal ramus side. The diapausing egg of *E. hawaiiensis* has a smooth surface but unlike the diapausing egg of *E. chihuahuaensis*, its shape is ovoid and pointed. In contrast to *E. ukera* and *E. chihuahuaensis* females of *E. hawaiiensis* deposit their eggs in clusters together with other females.

Description

Female: body illoricate, fusiform, gradually tapering into foot. Two conical toes with large spindle shaped foot glands. Corona with a tuft and two semicircles of cirri. Eyespot not visible. Trophi malleate. Fulcrum short, 1/2 of ramus length. Unci with 4–5 large teeth and 4–6 small teeth.

Male: body fusiform, tapering into foot. Digestive tract reduced. Mate guarding behavior as in *E. ukera* and *E. chihuahuaensis* was not observed in those rare cases that males appeared in cultures.

Measurements

Length of female 580–800 μm ; males not measured. Size of female subitaneous egg 123/127–138/162 μm (width/length). Size of diapausing egg 127/161–135/175 μm (width/length). Fulcrum length 21–26 μm . Ramus length 42–45 μm . Dorsoventral projection of ramus 470–590 μm^2 . Size of basal window 20–50 μm^2 . Length of basal window 8–13 μm , width of basal window 3–5 μm .

Distribution and ecology

The species has so far only been found in Lake Waiiau and not in other waters on the Hawaiian islands (Jersabek, 2003). Unlike other alpine lakes, Lake Waiiau is classified as hypereutrophic (Laws & Woodcock, 1981) and algal blooms were present when the species was found in the lake.

Etymology

The species is named after the type locality on the main island of the Hawaiian archipelago.

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