

MORPHOLOGICAL AND GENETIC COMPARISONS OF GOLDEN CRAYFISH,
ORCONECTES LUTEUS, AND RUSTY CRAYFISH, *O. RUSTICUS*,
WITH RANGE CORRECTIONS IN IOWA AND MINNESOTA

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A B S T R A C T

The distribution of the rusty crayfish, *Orconectes rusticus*, has been purported to include large portions of Iowa and Minnesota among many other states, whereas the golden crayfish, *O. luteus*, has been reported from Kansas, Arkansas, Missouri, and Illinois. Recent collections made in several river basins in Iowa and southern Minnesota and examination of museum specimens collected in Iowa revealed that many records attributed to *O. rusticus* are in fact *O. luteus*. We provide a suite of quantitative and qualitative characteristics, both morphological and genetic, that distinguish *O. luteus* and *O. rusticus* and also demonstrate that female *O. luteus* exhibit form alternation. Comparisons of mitochondrial 16S rRNA haplotypes among populations of *O. luteus*, *O. rusticus*, *O. cf. rusticus*, *O. placidus*, and *O. virilis* revealed relatively high levels of sequence divergence among taxa as well as within some taxa. We conclude that *O. luteus* is a native species in Iowa and southern Minnesota with introduced populations of *O. rusticus* occurring in both states. A more thorough survey of these states, particularly Iowa, is needed to assess the distributions of *O. rusticus* and *O. luteus*. If *O. rusticus* has a relatively restricted distribution in Iowa, efforts to control its anthropogenic and natural dispersal may be more effective if implemented in the near future. Our study indicates the need for more research in taxonomy, even in regions where the fauna is thought to be known well, in order for the conservation of native species and detection and management of nonindigenous species to be successful.

The rusty crayfish, *Orconectes rusticus* (Girard, 1852), has been studied intensively during the past two decades because of its ability to become established and to displace other crayfish species following its introduction to a new body of water (Capelli, 1982; Capelli and Munjal, 1982; Hobbs *et al.*, 1989; Olsen *et al.*, 1991). Introgressive hybridization has been either suspected or documented between *O. rusticus* and *O. propinquus* (Girard, 1852) in areas where *O. rusticus* has been introduced (Jezerinac, 1982; Perry *et al.*, 2001a, b). In addition to impacts on native crayfishes, nonindigenous rusty crayfish also might alter ecological food webs, resulting in a negative influence on natural fisheries (Capelli, 1982; Olsen *et al.*, 1991). Because of the detrimental effects of rusty crayfish on some aquatic ecosystems, it is important to identify the species accurately in

order to document its present and expanding distribution. The rusty crayfish occurs in Maine, Vermont, New Hampshire, Massachusetts, Connecticut, New York, Pennsylvania, West Virginia, North Carolina, Michigan, Illinois, Wisconsin, Minnesota, Iowa, New Mexico, Indiana, Kentucky, and Ohio, with portions of the latter three states constituting its native range (Crocker, 1979; Page, 1985; Hobbs, 1989; Alberstadt *et al.*, 1995; Jezerinac *et al.*, 1995; Daniels *et al.*, 2001; Fullerton and Watson, 2001). In Canada, it has been found in Ontario and Quebec (Corey, 1988; Dubé *et al.*, 2002).

The native distribution of the golden crayfish, *Orconectes luteus* (Creaser, 1933), includes portions of Kansas, Arkansas, Missouri, and Illinois, with the majority of its range in Missouri (Creaser, 1933; Williams, 1954; Pflieger, 1996; Minckley and Deacon, 1959;

Metcalf and Distler, 1961; Ghedotti, 1998; Poly and Wetzel, 2003). In the past, some populations of *O. luteus* in northeast Missouri and the Mississippi River bordering Illinois had been confused with either *O. rusticus* or *O. placidus* (Hagen, 1870) (Page and Burr, 1973; Page, 1985; Pflieger, 1996; Wetzel and Poly, 2000; Poly and Wetzel, 2003). Based on past taxonomic confusion involving *O. luteus* and *O. rusticus*, geographic distribution of *O. luteus* in Missouri and Illinois, and comments by Phillips (1979, 1980) about variability of *O. rusticus* in Iowa, we felt that at least some records of *O. rusticus* in Iowa and southern Minnesota (Phillips and Reis, 1979) could refer to *O. luteus*. Recent collections by us in Iowa and Minnesota and examination of museum specimens collected in Iowa confirmed our suspicions. Distinguishing characteristics and updated distributions are given for *O. luteus* and *O. rusticus* in Iowa and southern Minnesota, and we discuss the importance of taxonomy in regard to conservation of native species and management of nonindigenous species. We also demonstrate that female *O. luteus* undergo form alternation as shown recently for several other species of the genus *Orconectes* (Wetzel, 2002).

MATERIALS AND METHODS

We sampled eight sites in drainages where "variant" *O. rusticus* has been reported (*sensu* Phillips, 1979, 1980) on 10–11 May 2002 in central and eastern Iowa and three sites in eastern Iowa and southeastern Minnesota on 15–16 May 2002. Crayfishes were collected by hand and with a seine. Crayfishes were returned to the laboratory alive for color photography and extraction of abdominal muscle for genetic analyses, then preserved in 70% ethyl alcohol for morphological study and later deposited in the Museum of Comparative Zoology, Harvard University (MCZ). We borrowed additional specimens of Iowa "*O. rusticus*" from MCZ and the National Museum of Natural History (USNM), many of which were the specimens studied by Phillips (1979, 1980), and also used specimens and data from a previous study (Poly and Wetzel, 2003; see Material Examined), including specimens from the Illinois Natural History Survey (INHS) and from the Monte L. Bean Museum (Brigham Young University) Crustacean Collection (MLBM BYUC). Specimens were categorized to sex (male = M, female = F) and form (sexually competent = I, sexually incompetent = II) prior to taking measurements. Carapace length (CL) and the following chela measurements were recorded to the nearest 0.1 mm using digital calipers: chela length, chela palm width, chela depth, propodus width (= fixed finger; taken at middle of finger), dactylus width (taken at middle of finger), dactylus length, palm mesial margin length, and gap between fingers at widest point between closed fingers. Gonopod total length, mesial process length, and central projection length were measured on form I males. Width of the abdomen at pleonite 2 was recorded for female crayfish to test for the occurrence of

form alternation (Wetzel, 2002). We measured 274 *O. luteus* (86 MI, 42 MII, 106 FI, 40 FII) from Illinois, Iowa, Minnesota, and Missouri and 41 *O. rusticus* (16 MI, 4 MII, 19 FI, 2 FII) from Indiana, Iowa, and Ohio, excluding chelae that appeared to be regenerated.

We compared means of the following ratios using the Mann-Whitney *U* test: (1) dactylus length/palm mesial margin length (between *O. luteus* and *O. rusticus*, both sexes combined), (2) carapace length/pleonite 2 width (between female *O. luteus* [designated as form I or form II]), and (3) central projection length/gonopod total length (between form I males of *O. luteus* and *O. rusticus*). Results were considered significant at $\alpha = 0.05$. All statistics were performed with StatView 5.0 (SAS Institute, Inc., 1999).

Mandibles were examined with a dissecting microscope for qualitative differences in dentition, and some mandibles were examined with scanning electron microscopy (SEM) ($n = 2$ *O. luteus*, 2 *O. rusticus*). Mandibles were washed with detergent, sonicated, critical point dried, sputter coated with gold/palladium, and examined with a Hitachi S-570 SEM at 20 kV.

We sequenced a 528 base pair (bp) region of the mitochondrial 16S rRNA gene from the following species and sites: 23 *O. luteus* from Iowa (3 sites), 15 *O. luteus* from Minnesota (2 sites), 9 *O. rusticus* from Iowa (1 site), 17 *O. rusticus* from Indiana (1 site), 3 *O. rusticus* from Ohio (2 sites), 9 *O. cf. rusticus* from Kentucky (1 site), 1 *O. cf. rusticus* from Tennessee (1 site), 12 *O. virilis* (Hagen, 1870) from Iowa (4 sites), 31 *O. placidus* from Illinois (3 sites), and 6 *O. placidus* from Tennessee (2 sites) (Table 2). DNA methods followed those of Fetzner and Crandall (2003). Specimens of *O. rusticus* from Indiana and Ohio and specimens of *O. cf. rusticus* from Kentucky and Tennessee were included in the analyses for comparison to the Iowa material. Additionally, 39 distinct haplotype sequences from *O. luteus*, collected throughout the species range in Missouri and Illinois, were used to determine affinities of *O. luteus* material from Iowa and Minnesota (Fetzner and Crandall, 2003). A single specimen of *Cambarus monongalensis* Ortmann, 1905, from Pennsylvania was included as an outgroup.

Because levels of variation tend to be low within species and because reticulations (multiple connections) among haplotypes are possible, phylogenetic relationships are much better depicted as a network (Crandall *et al.*, 1994). The TCS v. 1.13 program was used to collapse multiple sequences into unique haplotypes and to construct haplotype networks for species (Clement *et al.*, 2000). A phylogenetic tree also was generated for the resulting haplotypes to show relationships among the species included in the analysis. The tree was produced using PAUP* v4.0b10 (Swofford, 2000) and the neighbor-joining (NJ) criterion with maximum-likelihood generated distances. The model of sequence evolution used to generate the likelihood distances was assessed using the program Modeltest v3.1 (Posada and Crandall, 1998). Confidence in nodes was assessed using the bootstrap with 1000 replicates (Felsenstein, 1985).

RESULTS

We collected four species of *Orconectes* in Iowa: 27 *O. luteus* (3 sites), 9 *O. rusticus* (1 site), 23 *O. virilis* (5 sites), and 1 *O. immunis* (Hagen, 1870) (1 site). *Orconectes luteus* was found in the Cedar and Wapsipinicon river drainages of east central Iowa (Otter and

Buffalo creeks, respectively). In Buffalo Creek, we caught *O. luteus*, *O. rusticus*, and *O. virilis*. *Orconectes luteus* also was collected in the Cedar River drainage of southeastern Minnesota (tributary of Little Cedar River and Otter Creek; Fig. 1), which lies about 400 air km to the north of other known native populations of *O. luteus* in northeastern Missouri.

Examination of museum specimens also revealed that previous records of *O. rusticus* in Iowa were based on *O. luteus* (Fig. 1; see Material Examined). *Orconectes rusticus* can be distinguished easily from *O. luteus* by coloration of live animals. *Orconectes luteus* possesses two dark saddles on the carapace (anteriorly and posteriorly), an orange-red tubercle at the base of the dactylus of the cheliped, orange-red dactyls on the walking legs, and an overall tan/light olive green body color (Fig. 2). *Orconectes rusticus* had a prominent rusty spot on each side of the posterolateral margins of the carapace, a rust-colored band down the center of the abdomen (dorsally), and an overall aquamarine greenish color that is most pronounced on the walking legs. The cephalic half of the incisor region on the mandibles of *O. luteus* has a crenate incisor margin versus the blade-like margin of *O. rusticus* (Fig. 3). The ratio dactylus length/palm mesial margin length was an excellent discriminator between *O. luteus* and *O. rusticus* (Mann-Whitney U , $P < 0.0001$) and provided good separation when displayed graphically (Fig. 4 A, B). The cephalic shoulder on the gonopod of form I males is well developed in *O. rusticus* and weakly developed in *O. luteus*. In addition, the ratio central projection length/gonopod total length differed significantly between form I males of the two species (Mann-Whitney U , $P = 0.0002$); however, the character was highly variable. The morphological and color characteristics that distinguish *O. luteus* and *O. rusticus* are summarized in Table 1.

Analysis of abdomen widths of female *O. luteus* revealed form alternation in the species. Form I (reproductive) females have wider abdomens than form II females (see Fig. 2 B, D), with the mean width of pleonite 2 differing significantly (Mann-Whitney U , $P < 0.0001$; Fig. 5). In addition to having wider abdomens, form I females either have conspicuous (white) glair glands (prior to egg laying) or are carrying eggs, larvae, or remnants of eggs attached to the pleopods; however, following the loss of larvae, form I females will exhibit none of the other

conditions for a period of time. Eight ovigerous females were collected in Otter Creek, Iowa, and color of the eggs was orange.

A total of 18 distinct haplotypes (2 *O. luteus*, 2 *O. rusticus*, 5 *O. cf. rusticus*, 3 *O. virilis*, 5 *O. placidus*, and 1 *C. monongalensis* (Genbank accessions: AF376483, AF376486, AY485432–AY485443, AY590472, AY609334–AY609338) were detected among the 127 new sequences examined in this study (Table 2). For *O. luteus*, two haplotypes were present in the Iowa and Minnesota populations, and these haplotypes matched those present in *O. luteus* from northeastern Missouri and westcentral Illinois (Fetzner and Crandall, 2003). These two haplotypes differed by only a single nucleotide substitution. The *Olu1* haplotype was found in all Iowa and Minnesota populations examined, except those from the tributary to Little Cedar River. The *Olu4* haplotype was found at high frequencies in the Iowa and Minnesota Otter creeks populations but was absent from the other sampled populations (although this haplotype may not have been detected due to small sample sizes).

For *O. rusticus* and *O. cf. rusticus*, we detected two and five different haplotypes, respectively, among the six populations included in the study. The dominant haplotype (*Oru1*) was found in all sampled populations of *O. rusticus*, all of which were north of the Ohio River (Iowa, Indiana, and Ohio). The *Oru2* haplotype was detected in a single individual from the Indiana population. Populations of *O. cf. rusticus* sampled from south of the Ohio River were found to be quite different from those of *O. rusticus*. The Kentucky *O. cf. rusticus* population contained four haplotypes and was quite distinct, differing from the Iowa, Indiana, and Ohio populations of *O. rusticus* by 3.9% average sequence divergence (p). Within the Kentucky population, we also found a single individual that contained a very unique haplotype, which was distantly related to the other haplotypes found at the same site as well as the populations of *O. rusticus* (Fig. 6). The Tennessee specimen was more closely related to populations of *O. rusticus* north of the Ohio River than it was to the Kentucky population of *O. cf. rusticus*, the latter being much closer geographically. This may suggest that the Kentucky population represents a new undescribed species, but additional sampling will be required from this area before a more definitive conclusion can be drawn.

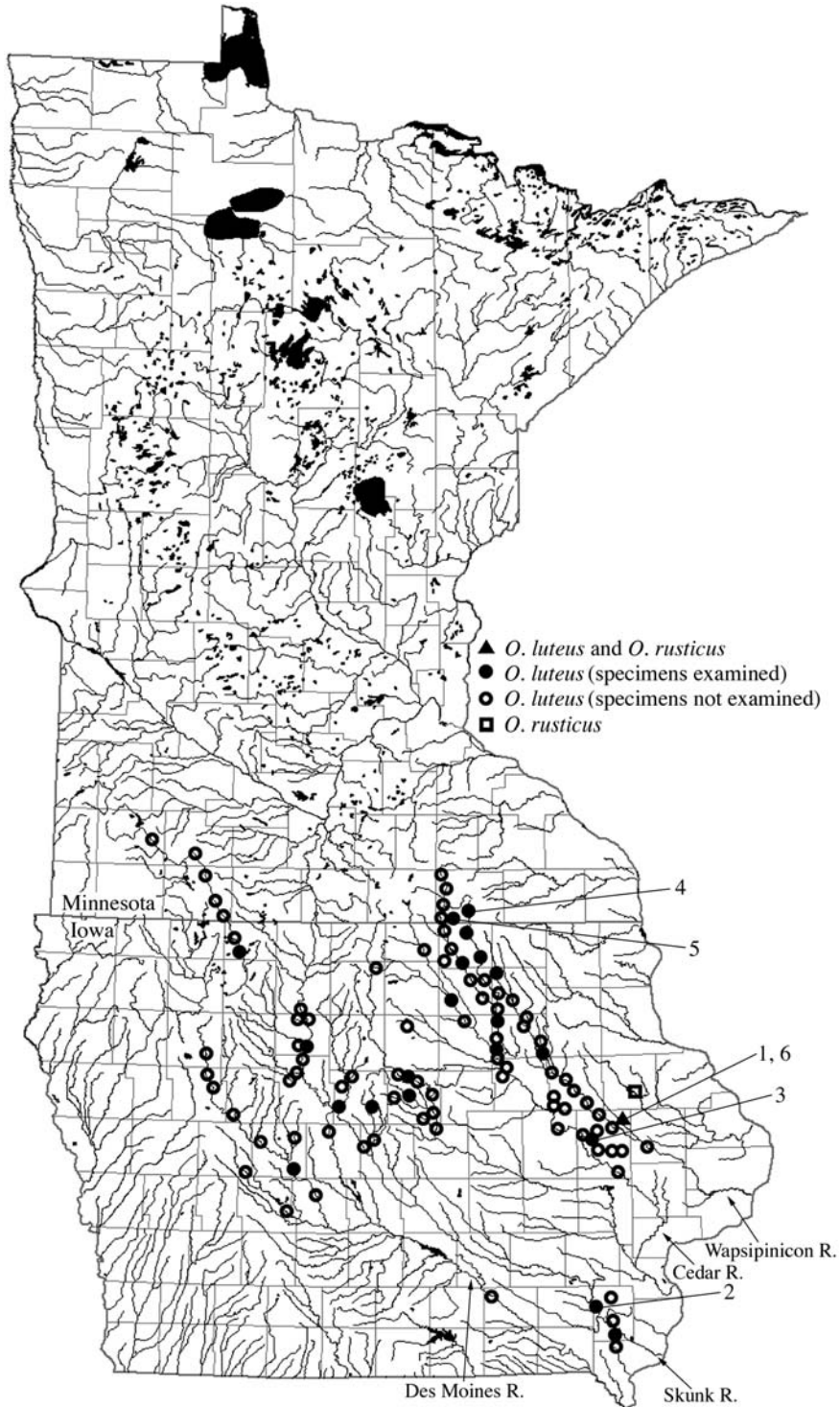


Fig. 1. Distribution of *Orconectes luteus* and *O. rusticus* in Iowa and southern Minnesota; *O. luteus*: solid circles (examined specimens, 21 sites), open circles (other "variant" *O. rusticus* [= *O. luteus*] reported in Phillips 1979, 1980, and Phillips and Reis, 1979, not examined, 78 sites); *O. luteus* and *O. rusticus* (sympatric): solid triangle (examined specimens, 1 site); *O. rusticus*: open squares (typical *O. rusticus* reported in Phillips 1979, 1980, not examined, 1 site). Numbered localities refer to Iowa and Minnesota sample sites for *O. luteus* and *O. rusticus* listed in Table 2.

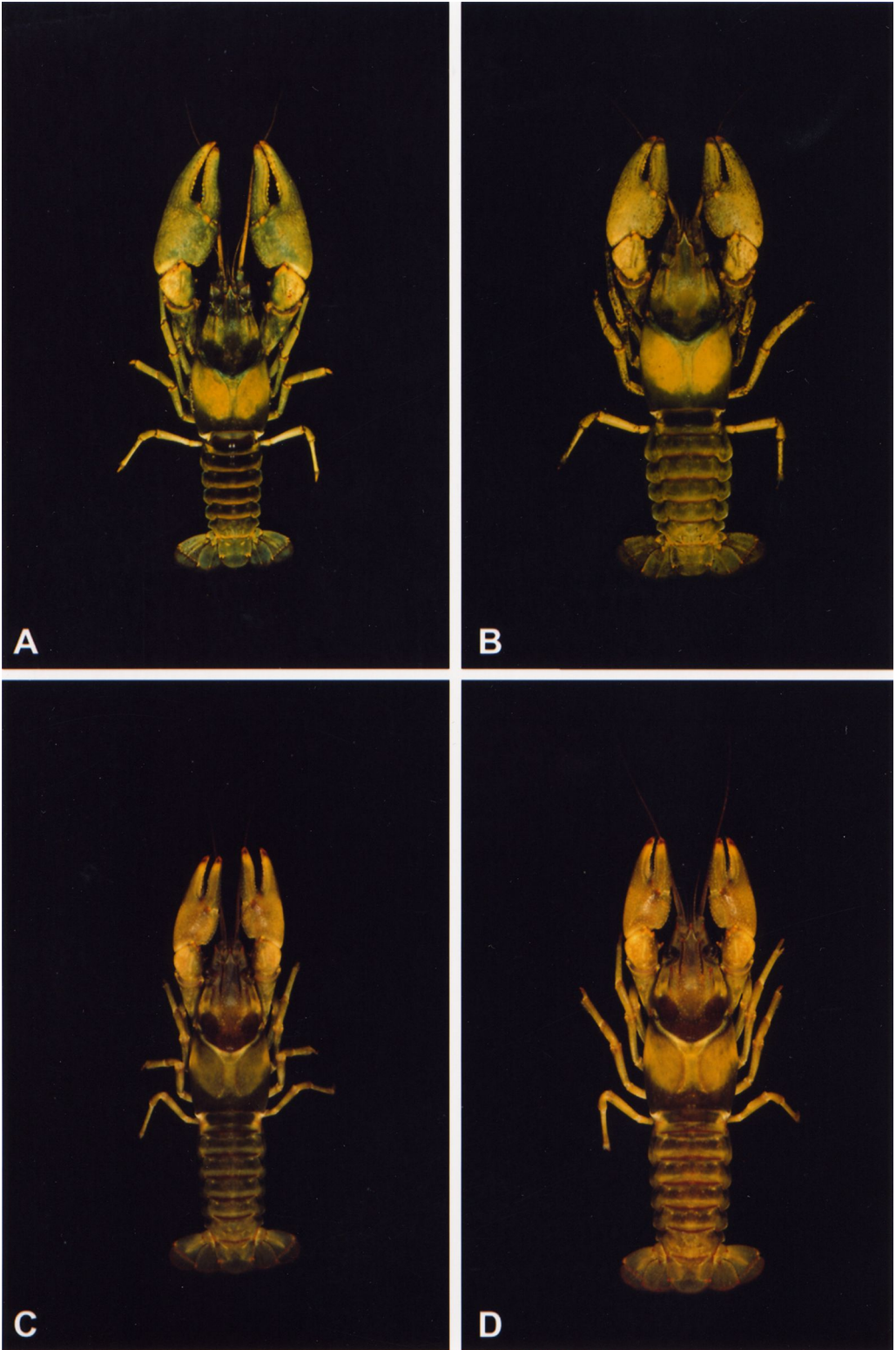


Fig. 2. Dorsal views of *Orconectes luteus* collected in Iowa and Minnesota. (A) 33.3 mm CL, MI; (B) 33.7 mm CL, FI; (C) 23.8 mm CL, MII; (D) 25.8 mm CL, FII. (A, B: MCZ 56210, Otter Cr., Minnesota; C, D: MCZ 56212, Otter Cr., Iowa.)

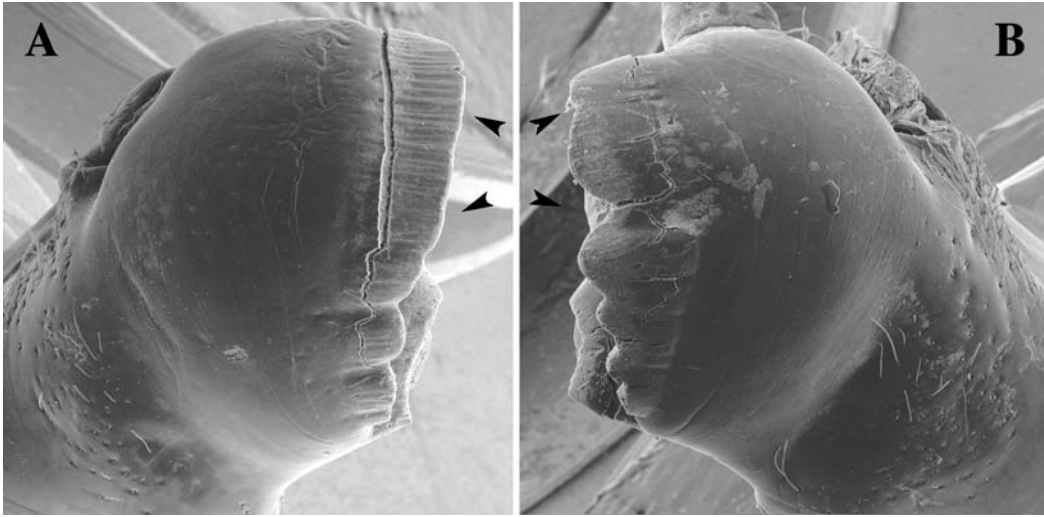


Fig. 3. Mandibles of (A) *Orconectes rusticus* and (B) *O. luteus*, showing blade-like incisor margin of *O. rusticus* and crenate incisor margin of *O. luteus* (A: 27.8 mm CL, MI, Tippecanoe River, Indiana; B: 28.3 mm CL, MI, Big River, Missouri).

Among the four *O. virilis* populations examined, we were able to detect three haplotypes that differed by 0.63% average sequence divergence (Fig. 6.). The *Ovi1* haplotype was found in Tipton Creek, Tributary to

Big Cedar Creek, and Otter Creek. Haplotype *Ovi2* was detected in Buffalo Creek and Otter Creek, whereas *Ovi3* was found only in Buffalo Creek.

Five haplotypes were detected among the examined populations of *O. placidus*. Population sampling of this species was similar to that of *O. rusticus* and *O. cf. rusticus* (northern vs. southern populations), thus allowing for a comparison of genetic variation over somewhat similar geographic distances. As was seen with *O. rusticus* and *O. cf. rusticus*, a clear separation of the *O. placidus* populations was observed between northern and southern populations. The southern Tennessee population (Cane Creek) was the most distinct, differing from other *O. placidus* populations by an average sequence divergence of 3.2%. The northern Tennessee population (Bledsoe Creek) only differed from Illinois populations by 1.1%.

The best fit model selected by the hierarchical likelihood ratio test was the HKY + I + G with t_i/t_v ratio = 3.3207, $I = 0.5169$, $G = 0.6103$, and base frequencies of $A = 0.3522$, $C = 0.2112$, $G = 0.1038$, $T = 0.3328$. Using these model parameters, all species were recovered as distinct monophyletic groups in the tree (except *O. cf. rusticus*), each with moderate to high levels of bootstrap support (Fig. 7). *Orconectes luteus* and *O. rusticus* haplotypes were quite distinct from one another, with an average sequence divergence of ~6.0% and an average of 31.7

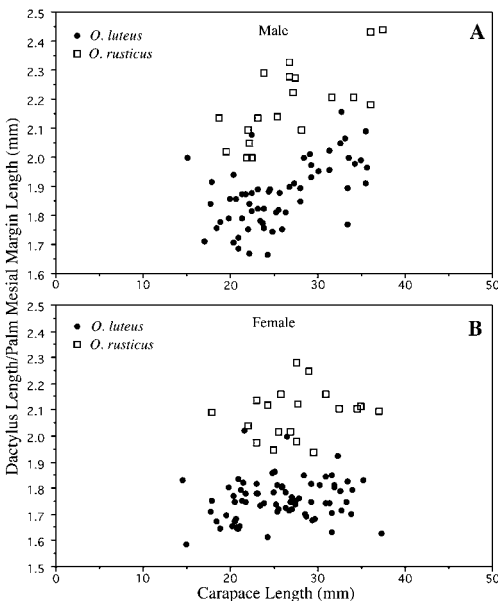


Fig. 4. Scatterplot of the ratio dactylus length/palm mesial margin length for male and female *Orconectes luteus* (mean = 1.809 ± 0.112 SD, $n = 134$) and *O. rusticus* (2.138 ± 0.124 , $n = 38$). Means differed significantly between species with both sexes included (Mann-Whitney U , $P < 0.0001$).

Table 1. Distinguishing characteristics for separation of *Orconectes luteus* and *O. rusticus*. Quantitative characters are given as range (mean \pm SD).

Characteristic	<i>O. luteus</i>	<i>O. rusticus</i>
Coloration ^a	Tan/light olive green base color. Two dark saddles on carapace. Orange-red tubercle at base of dactylus (dorsally) on cheliped. Small, white patches posterolaterally on carapace at junction with abdomen.	Overall aquamarine, greenish color most pronounced on walking legs. Rusty spot posterolaterally on each side of carapace. Rust-colored band down the center of abdomen (dorsally).
Mandible ^b	Cephalic half of incisor crenate.	Cephalic half of incisor blade-like.
Chelae ^c	Ratio of dactylus length/palm mesial margin length 1.588–2.160 (1.809 \pm 0.112).	Ratio of dactylus length/palm mesial margin length 1.940–2.443 (2.138 \pm 0.124).
Gonopod ^d	Shoulder weakly developed. Ratio of central projection length/gonopod total length of MI 0.298–0.407 (0.373 \pm 0.025).	Shoulder well developed. Ratio of central projection length/gonopod total length of MI 0.292–0.375 (0.338 \pm 0.025).

^a Refer to Fig. 2 (and Fig. 6 A, B in Poly and Wetzel (2003)); ^b refer to Fig. 3; ^c refer to Fig. 4; ^d even though the mean values differed significantly, the ratio central projection length/gonopod total length is quite variable

nucleotide substitutions (range 26–36). *Orconectes virilis* differed from *O. luteus* to a lesser degree than it did to *O. rusticus* + *O. cf. rusticus* (3.2% and 4.2%, respectively). *Orconectes placidus* grouped more closely with *O. rusticus* + *O. cf. rusticus* than it did to either *O. luteus* or *O. virilis*, differing from these species (or groups of taxa) on average by 4.1% (range 2.8%–5.4%), 4.9% (range 3.8%–5.4%), and 3.9% (range 3.4%–4.7%), respectively. All *Orconectes* spp. (with *O. rusticus* + *O. cf. rusticus*) were very distinct from *C. monongalensis*, with average sequence divergence values ranging from 9.1% to 9.5%. Interspecific hybridization was not indicated from the genetic data in this study.

DISCUSSION

Much of the confusion regarding *O. luteus* and *O. rusticus* is due to the overall similarity in body conformation and modest differences in the structure of the gonopods of form I males. We feel that in the area of crayfish taxonomy too much emphasis has been placed on form I gonopod structure at the expense of other useful characters. For example, the mandible is a reliable, qualitative character that distinguishes between *O. luteus* and *O. rusticus*. The blade-like incisor region of the mandible of *O. rusticus* has been noted and illustrated by Bouchard (1977, his fig. 2 d) and Hobbs and Jass (1988, their fig. 46 k). Bouchard (1977) listed *O. luteus* among the species possessing a blade-like incisor, which is contradictory to our observations; however, he included in his “blade-like” category many variations, some of which approach his “dentate-crenate” category. In our view, *O. luteus* mandibles are crenate

(= dentate-crenate), but regardless of what term is applied, the mandibles differ qualitatively between the two species and are useful for identification. Life colors of *O. rusticus* and *O. luteus* are quite different and reliable for separation of the two species (Fig. 2; Pflieger, 1996; Poly and Wetzel, 2003).

The genetic data clearly demonstrate that the two previously confused species, *O. luteus* and *O. rusticus*, also can be separated using mitochondrial gene sequence data (Fig. 7). These data also support the conclusion that *O. luteus* is present in drainages of Iowa and Minnesota, and that these populations are most closely related to populations in northeastern Missouri and an adjacent westcentral Illinois population (Apple Creek). The presence of *O. rusticus* in Iowa is supported by the genetic data with the Buffalo Creek samples grouping with specimens collected from Tippecanoe River, Indiana, and two different populations in Ohio.

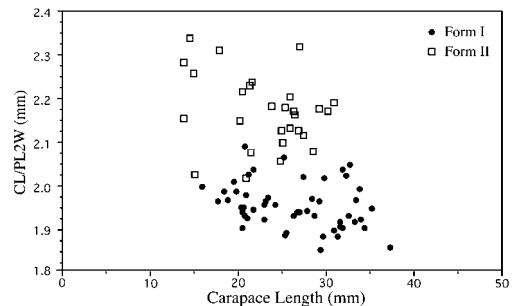


Fig. 5. Scatterplot of the ratio carapace length/pleonite 2 width (CL/PL2W), for form I (= reproductive form) (mean = 1.957 \pm 0.052 SD, n = 53) and form II female *Orconectes luteus* (2.172 \pm 0.084, n = 28). Means differed significantly between forms (Mann-Whitney U , P < 0.0001).

Table 2. Species, populations, and haplotype distributions (excluding Missouri and Illinois *Orconectes luteus* populations that appear in Fetzner and Crandall (2003)) for genetic samples examined in this study.

No.	Species	Locality	State	Sample size	Haplotype* (Abs. Frequency)
1	<i>Orconectes luteus</i>	Buffalo Creek at Hills Mill Road, Linn County	Iowa	1	<i>Olul</i> (1)
2	<i>Orconectes luteus</i>	Big Creek upstream of US Rt. 218, Henry County	Iowa	1	<i>Olul</i> (1)
3	<i>Orconectes luteus</i>	Otter Creek at E34 bridge in Toddville, Linn County	Iowa	21	<i>Olul</i> (1), <i>Olul4</i> (20)
4	<i>Orconectes luteus</i>	Tributary of Little Cedar River, Mower County	Minnesota	1	<i>Olul4</i> (1)
5	<i>Orconectes luteus</i>	Otter Creek at SR 6 bridge, Mower County	Minnesota	14	<i>Olul</i> (2), <i>Olul4</i> (12)
6	<i>Orconectes rusticus</i>	Buffalo Creek at Hills Mill Road, Linn County	Iowa	9	<i>Orul</i> (9)
7	<i>Orconectes rusticus</i>	Tippecanoe River at Hwy 18 bridge, Carroll County	Indiana	17	<i>Orul</i> (16), <i>Oru2</i> (1)
8	<i>Orconectes rusticus</i>	Stillwater River at SR 185, Webster, Darke County	Ohio	1	<i>Orul</i> (1)
9	<i>Orconectes rusticus</i>	Dry Fork Whitewater River, Hamilton County	Ohio	2	<i>Orul</i> (2)
10	<i>Orconectes cf. rusticus</i>	Powell River at mouth of Gap Creek, Claiborne County	Tennessee	1	<i>Oru7</i> (1)
11	<i>Orconectes cf. rusticus</i>	Middle Pitman Creek at bridge along SR 527, Taylor County	Kentucky	9	<i>Oru3</i> (5), <i>Oru4</i> (2), <i>Oru5</i> (1), <i>Oru6</i> (1)
12	<i>Orconectes virilis</i>	Buffalo Creek at Hills Mill Road, Linn County	Iowa	3	<i>Ovi2</i> (2), <i>Ovi3</i> (1)
13	<i>Orconectes virilis</i>	Tipton Creek at "N" Avenue, Hardin County	Iowa	3	<i>Ovi1</i> (3)
14	<i>Orconectes virilis</i>	Tributary to Big Cedar Creek at Cass Avenue, Henry County	Iowa	3	<i>Ovi1</i> (3)
15	<i>Orconectes virilis</i>	Otter Creek at E34 bridge in Toddville, Linn County	Iowa	3	<i>Ovi1</i> (2), <i>Ovi2</i> (1)
16	<i>Orconectes placidus</i>	Big Creek at CR 400E bridge, Hardin County	Illinois	9	<i>Opl1</i> (8), <i>Opl2</i> (1)
17	<i>Orconectes placidus</i>	Hogthief Creek at CR 3 bridge, Hardin County	Illinois	13	<i>Opl1</i> (13)
18	<i>Orconectes placidus</i>	Ohio River at Joppa boat ramp, Massac County	Illinois	9	<i>Opl1</i> (9)
19	<i>Orconectes placidus</i>	Bledsoe Creek at Hwy 231 bridge, Sumner County	Tennessee	3	<i>Opl3</i> (2), <i>Opl4</i> (1)
20	<i>Orconectes placidus</i>	Cane Creek at bridge along Boonshill Road, Lincoln County	Tennessee	3	<i>Opl5</i> (3)
21	<i>Cambarus monongalensis</i>	ditch (seepage area) along SR 4008, Greene County	Pennsylvania	1	<i>Com1</i> (1)
			Total	127	

* For *O. luteus*, the haplotypes detected in this study were identical to two haplotypes (1 and 4) listed by Fetzner and Crandall (2003). Therefore, haplotypes shown here for *O. luteus* have been numbered according to those matched from Fetzner and Crandall (2003) in order to avoid confusion when making comparisons across studies

Based on the genetic data, the introduced Iowa *O. rusticus* population appears to be derived from populations (Indiana and Ohio) within the native range of *O. rusticus* rather than from populations of *O. cf. rusticus* farther south in Kentucky and Tennessee. Fitzpatrick (1987) placed *O. luteus*, *O. placidus*, and *O. rusticus* in the subgenus *Procericambarus* and placed *O.*

virilis in the subgenus *Gremicambarus*. However, in this study, as in Crandall and Fitzpatrick (1996), relationships of 16S rRNA gene haplotypes of the species do not support the subgeneric assignments (Fig. 7).

Early reports of *O. rusticus* from West Fork Des Moines River, Iowa, by Faxon (1884, 1914) might have contributed to subsequent

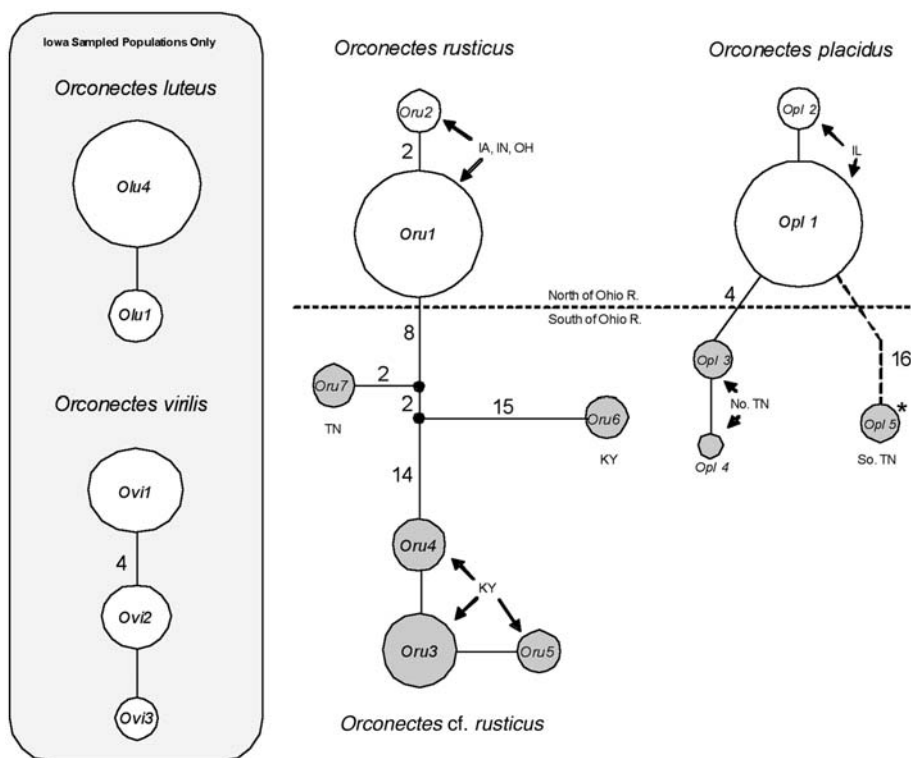


Fig. 6. Mitochondrial 16S haplotype networks for the species of *Orconectes* examined from Iowa, Minnesota, Illinois, Indiana, Ohio, Kentucky, and Tennessee. Size of circles is roughly proportional to the haplotype frequency in each species. Numbers indicate the number of mutational steps along the branches connecting each haplotype. If a number is missing on a branch, there is only a single mutational step. Haplotype designations are as in Table 2.

identifications of the species in the state; the specimens mentioned by Faxon (MCZ 7635) were examined in this study and determined to be *O. luteus*. Many other records of *O. rusticus* throughout Iowa were based on *O. luteus*, and Phillips (1979, 1980) indicated that most localities had the "variant" *O. rusticus* (= *O. luteus*). Because the samples we examined from localities across Iowa were all *O. luteus*, and Phillips (1979, 1980) was able to distinguish *O. luteus* from *O. rusticus*, most, if not all, of his localities probably refer to *O. luteus*. Because we did not examine specimens of *O. rusticus* from central and northern Minnesota, no records from these areas were included on Fig. 1. Additional sampling in Iowa and Minnesota and reexamination of other preserved specimens from the state will be necessary to determine the distributions of *O. luteus* and *O. rusticus* more precisely.

Phillips' (1979) color description of variant *O. rusticus* in Iowa agreed in detail with the coloration of *O. luteus* and initially spurred us to

collect specimens in Iowa. Helgen (1990) noted that *O. rusticus* in Minnesota had the blade-like incisor, except for specimens from the West Fork Des Moines River and Big Elbow Lake. She also noted that the West Fork Des Moines River specimens were brownish and lacked the rust-colored posterolateral spots on the carapace that are present on *O. rusticus*. Helgen's observations about the mandible and coloration of specimens from southern Minnesota are in agreement with our results; therefore, the statement in Helgen (1990: 11) that "The first dated collection of *O. rusticus* was in 1967 from Otter Creek, Lyle, Mower County," where we also collected *O. luteus*, almost certainly refers to *O. luteus*. However, the variation Helgen noted for Big Elbow Lake specimens of "*O. rusticus*" requires further investigation. Phillips (1979, 1980) collected typical *O. rusticus* only below Hartwick Lake dam (Maquoketa River dr.), but we also collected *O. rusticus* in Buffalo Creek (Wapsipinicon River dr.). Hobbs and Jass (1988) indicated that some *O. rusticus* varied in

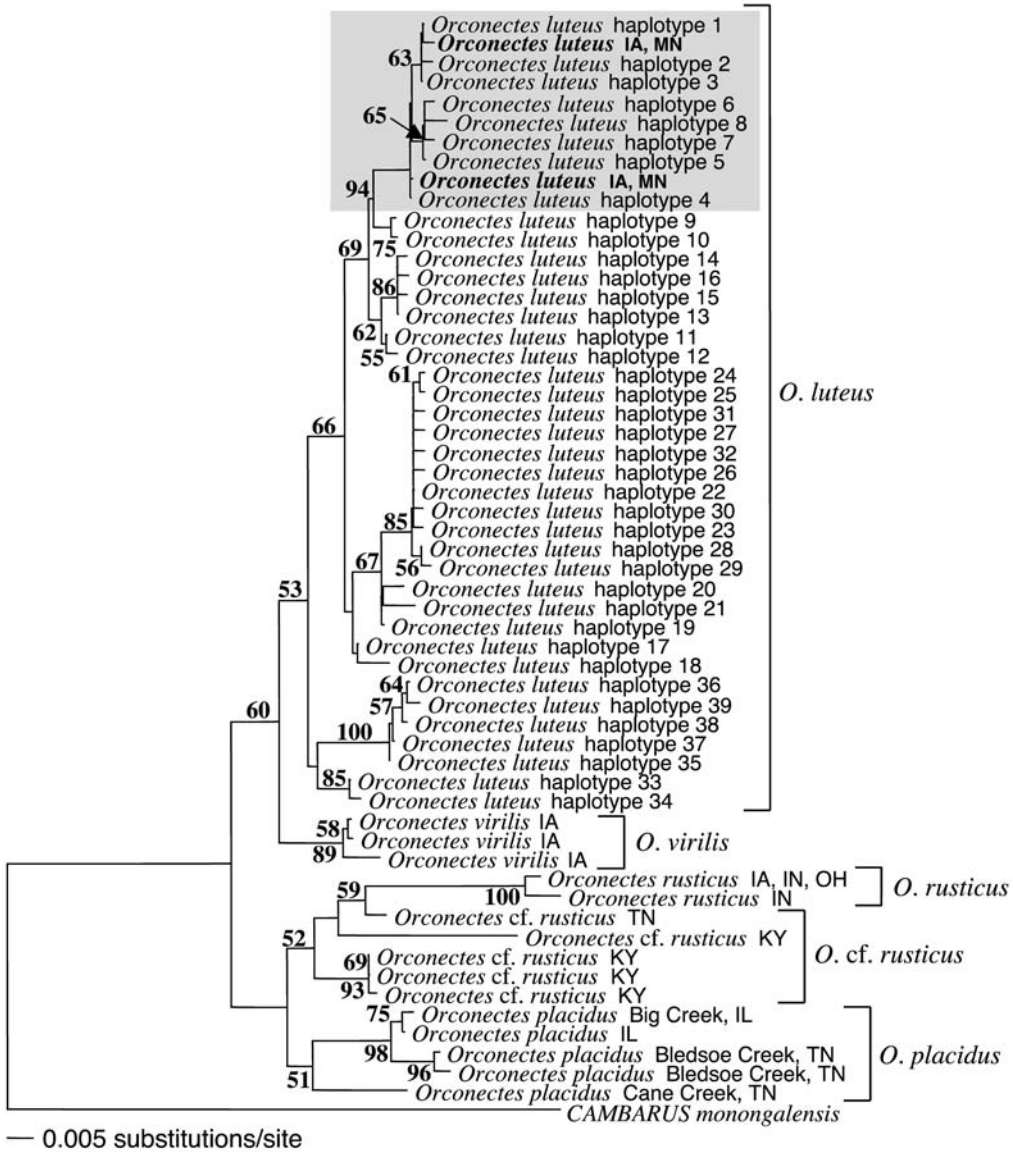


Fig. 7. Neighbor-joining tree depicting relationships among 16S haplotypes and species examined in this study. The shaded clade represents the northeastern Missouri and westcentral Illinois haplotype group, which also includes the populations from Iowa and Minnesota identified as *Orconectes luteus* in this study. Numbers at the nodes are bootstrap values from 1000 pseudoreplicates. The tree was rooted with *Cambarus monongalensis*.

such characters as the gonopod and mandible; thus, some records of *O. rusticus* from portions of Wisconsin and northern Illinois also could be *O. luteus* or another species. Other recent studies have clarified the range of *O. luteus* (Pflieger, 1996; Wetzel and Poly, 2000; Poly and Wetzel, 2003) and invalidated portions of the reported distributions of *O. rusticus* and *O. placidus* (Page and Burr, 1973; Page, 1985; U.S. Geological Survey, 1999). Neither we nor

Page (1985) have ever collected *O. rusticus* from the southern half of Illinois, contrary to the distributions given in Hobbs and Jass (1988) and Perry *et al.* (2001a).

Pflieger (1996) recommended sorting crayfishes in the field (or upon return to the laboratory) while they are still alive and preserving them in separate containers; this method allows life colors to be used for identification along with other characters. Taxonomic

research on crayfishes and dichotomous keys for identification would benefit from inclusion of a broader scope of characters from males and females of both forms, including color and morphometric data, rather than reliance primarily on gonopods and other characters of form I males.

Several studies have provided life history information about *O. luteus* (Phillips and Reis, 1979 [as *O. rusticus*]; Phillips, 1980 [as *O. rusticus*]; Pflieger, 1996; Muck *et al.*, 2002; DiStefano *et al.*, 2003), although female form alternation was not noted until now. Variation in *O. luteus* female form appears similar to that observed in *O. illinoiensis* Brown, 1956; *O. kentuckiensis* Rhoades, 1944; *O. indianensis* (Hay, 1896); *O. virilis*; and *O. cf. propinquus* as documented by Wetzel (2002). Female form alternation very likely occurs in most, if not all, *Orconectes* spp. and possibly other genera of crayfishes in which male form alternation is known. Variation in the annulus ventralis associated with form alternation in female *Orconectes* spp. may need to be accounted for before the annulus ventralis can be used reliably for species-level identification.

The river drainages in Missouri, Minnesota, Iowa, and Illinois, from which *O. luteus* has been collected are similar in that all the major rivers radiate as tributaries from the middle Mississippi River (Pflieger, 1996; Wetzel and Poly, 2000; Poly and Wetzel, 2003; Fig. 1). We consider *O. luteus* a native species in Iowa and Minnesota, whereas *O. rusticus* is an introduced species in both states. The distribution of *O. rusticus* could still be expanding as indicated in part by our collection in Buffalo Creek. This could be due to its own dispersal or via additional releases of specimens used for fishing bait.

Because nonindigenous species can impact native species and result in considerable economic costs, including losses through damage and costs of control measures (Pimentel *et al.*, 2000; Perry *et al.*, 2001b), accurate taxonomic information has become much more important for identification of native *versus* introduced species. At least 20 crayfish taxa, worldwide, have been introduced outside their native ranges, with varying degrees of ecological consequences (reviewed in Hobbs *et al.*, 1989). Effective conservation of native *O. luteus* and management of nonindigenous *O. rusticus* to prevent its expansion depend on defining their distributions accurately and on our ability to distinguish the two species.

Additional work, both genetic and morphological, is needed to help clarify our current understanding of the rusty crayfish, which poses a significant threat to native crayfish species wherever it has been introduced (Hobbs *et al.*, 1989; Perry *et al.*, 2001a). Specimens of "*O. rusticus*" from tributaries of the Ohio River in southern Indiana and Kentucky, including localities cited in Taylor (2000), exhibit morphological features and coloration that differ considerably from populations that we consider to represent the taxon *O. rusticus*, and might be confused with *O. rusticus* due to similarity of the gonopod (unpublished data). The morphological variation we observed and the genetic differences noted in this study indicate that *O. rusticus* as defined in earlier studies likely consists of multiple taxa. Currently, we are studying *O. rusticus* throughout its purported range to redefine the taxonomy and distribution of this and other species with which it has been confused.

MATERIAL EXAMINED

Orconectes luteus.—IOWA: MCZ 56207 ($n = 1$), Buffalo Creek at Hills Mill Road bridge, 2.5 km Southwest of Prairieburg, Linn Co., T86N, R5W, Sec. 29, 10 May 2002, J. E. Wetzel and W. J. Poly. MCZ 56209 ($n = 1$), Big Creek just upstream of U.S. Rt. 218 bridge, 3 km North of Mt. Pleasant, Henry Co., T72N, R6W, Sec. 28, SE 1/4, 16 May 2002, J. E. Wetzel. MCZ 56212 ($n = 25$), Otter Creek at Toddville Road (E34) bridge in Toddville, Linn Co., T84N, R8W, Sec. 12, SE 1/4 of SE 1/4, 10 May 2002, J. E. Wetzel and W. J. Poly. MINNESOTA: MCZ 56210 ($n = 24$), Otter Creek at State Route 6 bridge, 2 km Northeast of Lyle, Mower Co., T101N, R17W, Sec. 30/31 border, 15 May 2002, J. E. Wetzel. MCZ 56211 ($n = 1$), Tributary of Little Cedar River on west side of Adams [town], downstream of State Route 56 bridge at pedestrian bridge (old RR bridge), Mower Co., T101N, R16W, Sec. 11, 15 May 2002, J. E. Wetzel. ILLINOIS: INHS 2302 ($n = 1$), Mississippi River, Grand Tower, Jackson Co., T10S, R4W, Sec. 25, NW 1/4, 13 June 1981, M. Klutho, *et al.* INHS 2303 ($n = 1$), Mississippi River, Grand Tower, Jackson Co., T10S, R4W, Sec. 25, NW 1/4, 19 September 1981, M. Klutho, *et al.* INHS 2381 ($n = 1$), Mississippi River, 0.5 mi S Grand Tower, Jackson Co., T10S, R4W, Sec. 36, NW 1/4, 24 October 1985, W. Dimmick and B. R. Kuhajda. INHS 3582 ($n = 10$), pool, 3 mi SE Kaskaskia,

Randolph Co., T7S, R7W, Sec. 22, NW 1/4, 24 March 1975. INHS 3593 ($n = 5$), Mississippi River, mouth, Mary's River, Randolph Co., T7S, R6W, Sec. 33, SW 1/4, 19 March 1981, L. M. Page. INHS 3595 ($n = 1$), Mississippi River, RM 118.0–115.8, 2 mi NE Kaskaskia, Randolph Co., T6S, R7W, 13 July 1973, U.S. Army Corps of Engineers. INHS 7255 ($n = 2$), Mississippi River at Grand Tower (Devils Backbone Park), among rocks around old concrete boat ramp extending onto large sandy area, Jackson Co., T10S, R4W, Sec. 23, 7 October 1999, J. E. Wetzel, W. J. Poly, A. Miller, E. Poynter and J. Rush. INHS 7256 ($n = 2$), [same locality as INHS 7255], 31 October 1999, W. J. Poly and F. Kanekawa [one with a zebra mussel, *Dreissena polymorpha*, attached when collected]. INHS 9107 ($n = 1$), [same locality as INHS 7255], 13 April 2000, W. J. Poly [listed as MCZ uncataloged in Poly and Wetzel, 2003]. INHS 5181 ($n = 19$), Apple Creek, 3 mi E Haypress, Co. Rd. 1850N, Greene Co., T11N, R13W, Section 36, NE 1/4, 10 October 1995, C. A. Taylor and M. Pyron. MCZ 47134 ($n = 49$ [of 61]), Apple Creek, approximately 10 km NW of Carrollton, IL, Bluffdale Township, Greene Co., T11N, R13W, Sec. 36, NE 1/4, 6 November 1999, J. E. Wetzel and W. J. Poly. MLBM BYUC uncataloged ($n = 21$), [same locality as MCZ 47134 above], 21 November 1999, W. J. Poly and F. Kanekawa. INHS 9106 ($n = 1$), Mississippi River along riprap covered bank near boat ramp, approximately 1 km downstream of State Route 51/150 bridge (and River Mile 109.5), Randolph Co., T7S, R7W, Sec. 24, SW 1/4, 13 November 1999, J. E. Wetzel and W. J. Poly. INHS 9104 ($n = 1$), [same locality as INHS 9106 above], 27 November 1999, W. J. Poly and F. Kanekawa [with 4 zebra mussels, *Dreissena polymorpha*, attached when collected]. MCZ 47144 ($n = 1$), [same locality as INHS 9106 above], 13 April 2000, W. J. Poly. MCZ 47145 ($n = 1$), [same locality as INHS 9106 above], 6 October 2001, J. E. Wetzel and W. J. Poly. MCZ 47142 ($n = 1$), Mississippi River near mouth of Marys River, along upstream side of first wingdam below mouth of Marys River, Randolph Co., T7S, R6W, Sec. 32, NE 1/4, 13 November 1999, J. E. Wetzel and W. J. Poly. MCZ 47131 ($n = 4$), Mississippi River adjacent wingdam 200 m upstream of confluence with tributary stream, 7.5 km N of Grand Tower, Fountain Bluff Township, Jackson Co., T9S, R4W, Sec. 34, 9 December 2000, J. E. Wetzel and W. J. Poly [one with a zebra mussel,

Dreissena polymorpha, attached]. INHS 9100 ($n = 4$), Mississippi River, wingdam adjacent to Miller City Road, Alexander Co., T16S, R2W, Sec. 25, 12 August 2001, J. E. Wetzel and W. J. Poly. INHS 9103 ($n = 2$), Mississippi River, 1.2 km N of Thebes, Alexander Co. T15S, R3W, Sec. 8, 6 January 2002, W. J. Poly and J. E. Wetzel. Uncataloged ($n = 2$ juv., no voucher, specimens died in captivity), [same locality as INHS 9103 above], 20 May 2000, J. E. Wetzel and W. J. Poly. MISSOURI: INHS 9105 ($n = 5$), Big River at State Route 8 bridge, approximately 5 km NE of Irondale, St. Francois Co., T36N, R4E, Sec. 6, NW 1/4, 8 October 1999, J. E. Wetzel and W. J. Poly. Uncataloged ($n = 4$), [same locality as INHS 9105 above], 13 April 2000, J. E. Wetzel and J. Bartletti. MLBM BYUC 02–08 ($n = 26$), INHS 9111 ($n = 21$), Whitewater River at K Road bridge, 2.5 km NNW of Sedgewickville, Bollinger Co., T33N, R10E, near Sec. 22, 16 December 1999, J. E. Wetzel and W. J. Poly. USNM 98344 ($n = 1$, holotype), USNM 98345 ($n = 1$, allotype), Niangua River at mouth of Greasy Creek, 5 mi SE Buffalo, Dallas Co., 28 August 1931, J. C. Saylor. USNM 69347 ($n = 3$, paratypes), Rubidoux Creek, Waynesville, Pulaski Co., 22 August 1929, E. P. Creaser and E. B. Williams. USNM 117130 ($n = 43$, paratypes), Johnson Creek near Halltown, Laurence Co., 31 August 1930, E. P. Creaser. USNM 117137 ($n = 7$, paratypes), Roubidoux Creek, Pulaski Co., 22 August 1929, E. B. Williamson. USNM 117138 ($n = 3$, paratypes), headwaters of Niangua River near Marshfield, Webster Co., no date, J. C. Saylor.

Orconectes luteus (reidentified specimens).— IOWA: MCZ 7635 ($n = 3$), West Fork of Des Moines River, Spring Vale, Humboldt Co., [no date], F. W. Putnam and C. Cooke. USNM 148520 ($n = 6$), Little Cedar River, Riverview Park at Stacyville, Mitchell Co., T100N, R16W, Sec. 31, 23 July 1977, G. and L. Phillips. USNM 148470 ($n = 2$), Cedar River, North Cedar Park, 1 mi E of Plainfield, Bremer Co., T93N, R14W, Sec. 20, 2 June 1977, G. S. Phillips. USNM 148523 ($n = 2$), Little Cedar River, 4 mi. S/1 mi W of New Haven, Mitchell Co., T97N, R15W, Sec. 18, 24 July 1977, G. and L. Phillips. USNM 148494 ($n = 3$), South Fork Iowa River, Logsdon Park, 9 mi S of Iowa Falls, Hardin Co., T88N, R21W, Sec. 35, 11 June 1977, G. and L. Phillips. USNM 148541 ($n = 2$), [North] Raccoon River, Rippey Fishing

Access, 4 mi W/2 mi. S of Rippey, Greene Co., T82N, R29W, Sec. 19, 21 August 1977, G. and L. Phillips. USNM 148516 ($n = 4$), Shell Rock River, 2 mi S/3 mi W of Waverly, Bremer Co., T91N, R14W, Sec. 18, 13 July 1977, G. S. Phillips. USNM 176570 ($n = 1$), Shell Rock River, 3 mi W/3 mi N of Marble Rock, Floyd Co., T95N, R18W, Sec. 35, 13 July 1977, G. Phillips. USNM 176553 ($n = 2$), Boone River, 4 mi N/3 mi W of Stanhope, Hamilton Co., T87N, R26W, Sec. 14, 11 June 1977, G. and L. Phillips. USNM 148472 ($n = 1$), Wapsipinicon River, Goodale Conservation Area, 2 mi S/5 mi W Alta Vista, Chickasaw Co., T97N, R14W, Sec. 29, 2 June 1977, G. S. Phillips. USNM 176627 ($n = 2$), Bougus [Bogue] Creek, 5 mi E of Salem, Henry Co., T70N, R6W, Sec. 27, 24 May 1978, G. and L. Phillips. USNM 148442 ($n = 1$), [South] Skunk River, at Jewell, Lyon Twp., Hamilton Co., T87N, R24W, Sec. 34, 15 April 1977, G. S. Phillips. USNM 148526 ($n = 5$), East Fork Des Moines River, S edge of Dakota City, Humboldt Co., T91N, R28W, Sec. 7, 31 July 1977, G. and L. Phillips. USNM 176571 ($n = 3$), Cedar River, Osage Spring Park, Mitchell Co., T98N, R17W, Sec. 27, 24 July 1977, G. and L. Phillips. USNM 148493 ($n = 6$), Iowa River at Iowa Falls, Hardin Co., T89N, R20W, Sec. 20, 11 June 1977, G. and L. Phillips. USNM 148499 ($n = 3$), Wapsipinicon River, 2 mi S/3 mi E of Readlyn, Bremer Co., T91N, R11W, Sec. 20, 15 June 1977, G. S. Phillips. USNM 176652 ($n = 1$), West Fork Des Moines River at Wallingford, Emmett Co., T98N, R33W, Sec. 6, 1 July 1978, G. Phillips. USNM 176600 ($n = 10$), Shell Rock River, 2 mi S/2 mi W of Waverly, Bremer Co., T91N, R14W, Sec. 28, 26 April 1978, G. Phillips.

Orconectes rusticus.—OHIO: MCZ 47141 ($n = 2$), Stillwater River, 100 m upstream of State Route 185 bridge at Webster, 4 km South of Versailles, Darke Co., T10N, R4E, Sec. 31, NE 1/4, 3 September 2001, W. J. Poly and A. J. Paulus. Uncataloged ($n = 6$), Dry Fork White-water River at New Haven Road bridge, Hamilton Co., 11 September 2003, J. E. Wetzel. INDIANA: MCZ 56214 ($n = 25$), Tippecanoe River at Highway 18 bridge, Carroll Co., Sec. 21, SW 1/4, SW 1/4, 5 September 2002, J. E. Wetzel. Uncataloged ($n = 5$), [same locality as MCZ 56214, Tippecanoe R.], 11 October 1999, J. E. Wetzel and R. M. Wetzel. IOWA: MCZ 56213 ($n = 9$), Buffalo Creek at Hills Mill Road bridge, 2.5 km Southwest of Prairieburg, Linn

Co., T86N, R5W, Sec. 29, 10 May 2002, J. E. Wetzel and W. J. Poly.

Orconectes virilis.—IOWA: MCZ 56216 ($n = 3$) [collected with MCZ 56207 and MCZ 56213 above], MCZ 56217 ($n = 3$) [collected with MCZ 56212 above]. Uncataloged ($n = 10$), Tributary to Big Cedar Creek at Cass Avenue, 7.5 km S of Rome, Henry Co., T71N, R7W, Sec. 32, 10 May 2002, J. E. Wetzel and W. J. Poly. Uncataloged ($n = 6$), Tipton Creek at "N" Avenue, 6 km NW of New Providence, Hardin Co., T87N, R20W, Sec. 30, 11 May 2002, J. E. Wetzel and W. J. Poly.

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