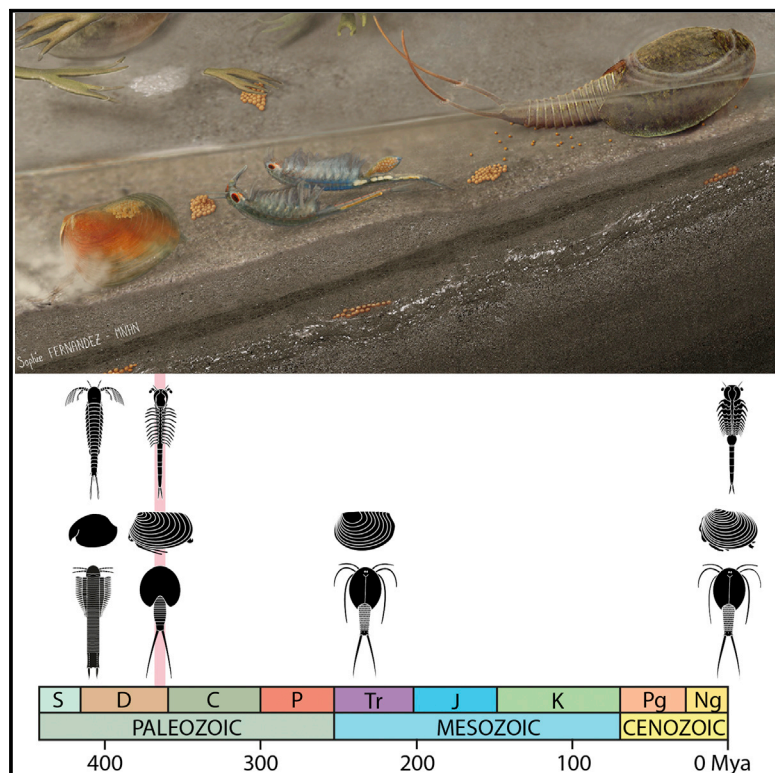


Current Biology

A 365-Million-Year-Old Freshwater Community Reveals Morphological and Ecological Stasis in Branchiopod Crustaceans

Graphical Abstract



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In Brief

Gueriau et al. describe a 365-million-year-old ephemeral pool community composed of the earliest modern-looking branchiopod crustaceans. Preservation of drought-resistant eggs illustrates how these animals colonized temporary terrestrial environments, leading to remarkable ecological and morphological stasis that persists to the present day.

Highlights

- A branchiopod community is described from 365-million-year-old freshwater sediments
- It includes the earliest modern-looking anostracan, notostracan, and spinicaudatan
- Resting eggs allowed them to colonize and to survive in terrestrial environments
- Their remarkable ecological and morphological stasis is discussed

A 365-Million-Year-Old Freshwater Community Reveals Morphological and Ecological Stasis in Branchiopod Crustaceans

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SUMMARY

Branchiopod crustaceans are represented by fairy, tadpole, and clam shrimps (Anostraca, Notostraca, Laevicaudata, Spinicaudata), which typically inhabit temporary freshwater bodies, and water fleas (Cladocera), which live in all kinds of freshwater and occasionally marine environments [1, 2]. The earliest branchiopods occur in the Cambrian, where they are represented by complete body fossils from Sweden such as *Rehbachella kinnekullensis* [3] and isolated mandibles preserved as small carbonaceous fossils [4–6] from Canada. The earliest known continental branchiopods are associated with hot spring environments [7] represented by the Early Devonian Rhynie Chert of Scotland (410 million years ago) and include possible stem-group or crown-group Anostraca, Notostraca, and clam shrimps or Cladocera [8–10], which differ morphologically from their modern counterparts [1, 2, 11]. Here we report the discovery of an ephemeral pool branchiopod community from the 365-million-year-old Strud locality of Belgium. It is characterized by new anostracans and spinicaudatans, closely resembling extant species, and the earliest notostracan, *Strudops goldenbergi* [12]. These branchiopods released resting eggs into the sediment in a manner similar to their modern representatives [1, 2]. We infer that this reproductive strategy was critical to overcoming environmental constraints such as seasonal desiccation imposed by

living on land. The pioneer colonization of ephemeral freshwater pools by branchiopods in the Devonian was followed by remarkable ecological and morphological stasis that persists to the present day.

RESULTS

The fossils described herein come from the Late Famennian (VCo Opper *Rugospora radiata* interval biozone) locality of Strud, Belgium [13]. The depositional environment is characterized by channel filling layers that preserve distinct but synchronous continental fossil assemblages (Figure 1). Isolated remains of sarcopterygians, including early tetrapods, occur in association with placoderms [14–17], actinopterygians, acanthodians, and plant macrofossils [18] in yellow to brown arkosic sandstones deposited in a flood channel. Complete early decapods [19, 20], eurypterids, a putative insect [21–23], and plant microfossils including first seeds [18] are present in black to green shales, indicating a low-energy, restricted floodplain habitat. The fossil branchiopods (Figures 2 and S1A), including *Strudops goldenbergi*, the earliest unequivocal representative of total-group Notostraca [12], were recovered from small (a few tens of centimeters in extension) lenses of fine, dark gray shale lying on a dark siltstone exhibiting millimetric sandy laminae. These lenses also yielded plant microfossils but lack vertebrate remains [13]. The nature of the sediments indicates that deposition took place in fresh to brackish shallow-water pools that periodically dried out: this was the restricted ephemeral environment inhabited by the crustacean community, as for recurrent associations of anostracans, notostracans, and spinicaudatans today [2, 11]. Here we describe *Haltinnaias serrata* and *Gesvesia pernegrei*, the first unequivocal representatives of total-group Anostraca

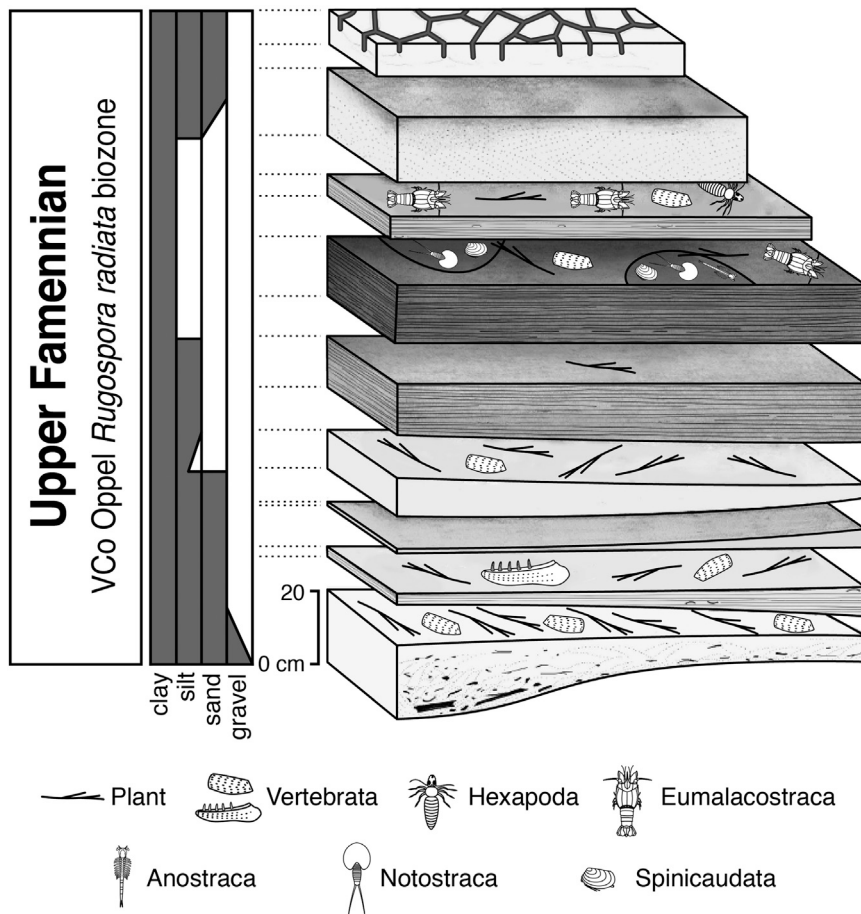


Figure 1. Stratigraphy of the Strud Channel Filling Deposits Showing Their Main Fossiliferous Content (Late Devonian, Belgium)

Schematic block diagram detailing the 1.2-m-thick fining-upward channel filling succession that yielded the fossils. In the field, beds are inverted with a dip of 80° to the south and represent only the upper part of a section beginning in Lower Famennian marine sediments. The sequence shallows upward from a channel fill through planar-laminated beds to desiccation-cracked sandstones. Fossils are not to scale. Modified from [13].

and S2G); abdomen with genital segment bearing forward-facing abdominal spines (Figures 2B, 2D, 2E, 2F, S2D, and S2F), at least six apodous segments, and telson bearing cercopods (Figures 2A–2F); female with longer (as long as the abdomen width) abdominal spines and a protruding brood pouch with a series of small distal spines (Figures 2A, 2C, S2C, and S2E); male with long, fine posteriorly directed forceps-shaped antenna (Figures 2E, 2F, and S2H). A small, dark spherical structure medial to the head may represent a nuchal organ (Figure S2G).

Remarks. Sexual dimorphism is marked in living anostracans: the male antennae are modified into large claspers to grasp the female during copulation, and females have two genital segments forming a

ventral brood pouch where fertilization and formation of eggs occur [24, 25] (Figures S1B and S1C). The co-occurrence, similar size, and small number of male and female anostracan specimens recovered from the Strud locality indicate that they likely belong to the same species.

Systematic Paleontology

The New Anostracan

Arthropoda von Siebold, 1848.

Branchiopoda Latreille, 1817.

Anostraca Sars, 1867.

Haltinnaias serrata gen. et sp. nov.

Etymology. The genus name is from “Haltinne,” the district including the Strud locality, and “naias,” a type of water nymph (gender feminine). The species name is from “serratus,” referring to the series of ventral spines on the brood pouch.

Material. Holotype IRSNB a 12928a, b (female, part and counterpart; Figures 2A–2D and S2A–S2F); paratypes IRSNB a 12930 (male, part only; Figures 2E and 2F), IRSNB a 12929 (part only; Figure S2G), and IRSNB a 12931a, b (part and counterpart; Figure S2H), from Strud, Gesves municipality, Belgium (50°26′43.32″N, 5°03′24.86″E).

Diagnosis. Anostracan with long, forward-facing abdominal spines. Female with a brood pouch with a series of small distal spines.

Description. Distinct oval head bearing pedunculate eyes; thorax with 11 pairs of thoracopods bearing setae (Figures 2E, 2F,

and S2G); abdomen with genital segment bearing forward-facing abdominal spines (Figures 2B, 2D, 2E, 2F, S2D, and S2F), at least six apodous segments, and telson bearing cercopods (Figures 2A–2F); female with longer (as long as the abdomen width) abdominal spines and a protruding brood pouch with a series of small distal spines (Figures 2A, 2C, S2C, and S2E); male with long, fine posteriorly directed forceps-shaped antenna (Figures 2E, 2F, and S2H). A small, dark spherical structure medial to the head may represent a nuchal organ (Figure S2G).

The New Spinicaudatan

Arthropoda von Siebold, 1848.

Branchiopoda Latreille, 1817.

Spinicaudata Linder, 1945.

Gesvesia pernegrei gen. et sp. nov.

Etymology. The genus name is from “Gesves,” the county where the locality occurs (gender feminine). The species name honors Vincent N. Pernègre (MNHN, Paris), who actively contributed to fossil collection in the field.

Material. Holotype IRSNB a 12932 (part only; Figure 2G); paratypes IRSNB a 12934a, b (part and counterpart; Figures 2H and 2I), IRSNB a 12936 (part only; Figures 2J–2L), IRSNB a 12933 (part only; Figure S2I), and IRSNB a 12935a, b (part and counterpart; Figures S2J and S2K), from the same locality as *H. serrata*.

Diagnosis. Spinicaudatan with limnadiform carapace with length/height ratio of 1.6, straight hinge line about 2/3 the total length of the carapace, and anterior and posterior cardinal angles 130° and 120°, respectively.

Description. Mean length/height ratio of the bivalved carapace 1.63 ± 0.39; umbo unpronounced, not connected to the hinge line; first larval valve unornamented; numerous growth lines

forming narrow concentric ribs on the entire surface of the valve and converging at the anterior cardinal angle; growth lines more closely spaced further from this angle (Figure 2G). Details of the preserved internal anatomy include a well-defined head, thorax, and abdomen (Figures 2H and 2I), the head occupying much less than 50% of the body; long second antennae and long mandibular molar process (Figure S2I); conical telson with curved posteriorly facing cercopods (Figures 2H, 2I, S2J, and S2K); spiral intestinal tract; first antennae and thoracopods poorly preserved. Some individuals display small clusters of dark, 163- μm spherical structures under the carapace, interpreted as resting eggs (Figures 2J and 2K).

Remarks. Except for a few well-preserved examples from the Carboniferous [26, 27], bivalved branchiopod fossils usually preserve carapaces only, which display highly homoplastic features that provide little systematic information. The carapace morphology of *G. pernegrei* is typical of Spinicaudata [28], and preservation of the body within the carapace in some specimens shows a small head and a relatively long trunk (Figures 2H, 2I, S2J, and S2K), proportions similar to those in extant Spinicaudata (Figure S1D) and clearly different from extant Laevicaudata.

Resting Eggs

Specimens of the spinicaudatan *G. pernegrei* and the notostracan *S. goldenbergi* display small clusters of dark spherical structures under the carapace (Figures 2J–2L, 3E, 3F, and S3C–S3E). These structures have a mean size of 163 μm in *G. pernegrei* and 71 μm in *S. goldenbergi* (see measurements in Figure S4). In *G. pernegrei*, they lie dorsal to the trunk (Figures 2J and 2L). Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) reveals that these structures are composed of carbon and calcium (Figures 2L and 2M), in contrast to the sedimentary matrix, which is composed dominantly of silicon, oxygen, aluminum, potassium, magnesium, and iron. The presence of calcium is presumably a result of authigenic mineralization after burial. In *S. goldenbergi*, several clusters lie in the thoracopod region (Figures 3E, 3F, and S3C–S3E). The shape, size, and position of these structures inside the crustaceans, and their organization in clusters, are typical of the drought-resistant eggs (and egg pouches) of some related extant branchiopods [1, 2] (Figures 3D and S1B–S1E). Their preservation in three dimensions (Figure 3C), which contrasts with the two-dimensional preservation of other features, indicates that they were decay resistant, and we interpret them as resting eggs. Accumulations of larger resting eggs (mean size 149 μm) as an egg bank in the sediment (Figures 3A, 3B, S3A, and S3B) are attributed to *G. pernegrei* rather than to *S. goldenbergi*, whose 71- μm eggs have been observed only within the carapace (see size comparisons in Figure S4).

DISCUSSION

Affinities of the Strud Branchiopods

The soft anatomy of *Strudops goldenbergi* identifies it as the earliest unequivocal member of total-group Notostraca [12]. Although *Haltinaias serrata* and *Gesvesia pernegrei* can be confidently assigned to total-groups Anostraca and Spinicaudata, respectively, it is difficult to determine their phylogenetic relationships within these clades. Attempts to determine phylo-

genetic relationships between extant and fossil spinicaudatans have been initiated [28], but many points remain to be clarified, and it is not possible to confidently assign *G. pernegrei* to any subclade of Spinicaudata.

H. serrata has pedunculate eyes and non-locomotory uniramous antennae (Figures 2A–2F and S2A–S2H), characters shared by all anostracans except the Early Devonian *Lepidocaris rhyniensis* [8]. The systematics of extant anostracans is based on the structure and shape of the legs, gonopod, brood pouch, and male head [24, 25], characters that are rarely preserved in fossils. Only one known fossil anostracan can be assigned to an extant family (Branchinectidae), *Branchinecta barstowensis* from the Miocene of California (USA) [29]. The male of *H. serrata* bears a simple antenna without any cephalic expansion (it is not a modified frontal appendage). This configuration is found in Chirocephalidae and Branchinectidae (e.g., in *Linderiella* and *Branchinecta* [24, 25]). Spines on the abdomen are present in Streptocephalidae and Chirocephalidae (e.g., in *Streptocephalus* and *Chirocephalus* [24, 25]), but such homoplastic characters cannot be used to assign *H. serrata* to any extant family. The presence of a series of small distal spines on the brood pouch of female *H. serrata* is the most diagnostic character, but it is not shared by any other extant or fossil anostracan.

Morphological and Ecological Stasis in Ephemeral Pool Branchiopods at Least since the Late Devonian

One outcome of our results is to show that the composition of the branchiopod community associated with freshwater ephemeral pools (Anostraca, Notostraca, and Spinicaudata) persisted over at least 365 million years. Also remarkable is the striking resemblance between the Late Devonian fossils and their extant relatives, reflecting long-term morphological stasis (Figure 4). The oldest previously known modern-looking notostracans and anostracans are from the Carboniferous of Germany [30, 31] and the Jurassic of Mongolia [32, 33], respectively. The Strud fossils indicate an earlier origin, confirming the evidence of time-calibrated phylogenetic trees [34, 35]. Furthermore, the branchiopod community itself, also dominated by Anostraca, Notostraca, and Spinicaudata in ephemeral pools today [1, 2, 11], experienced remarkable ecological stasis. Such ephemeral pool communities are rare in the fossil record. Except for an example from the Cretaceous of Russia that includes Laevicaudata in addition [36, 37], other occurrences are confined to Notostraca and Spinicaudata, notably in the Permian [38, 39] and Triassic [40] of France. The absence of Anostraca probably reflects their lower fossilization potential due to their delicate nature and lack of a carapace. The freshwater community from the well-known Early Devonian Rhynie Chert of Scotland is significantly different, with a depositional environment interpreted as hot-spring shallow ponds [7]. This community comprises at least three species of Branchiopoda, *Lepidocaris rhyniensis*, *Castracollis wilsonae*, and *Ebulliticaris oviformis*, which are considered to represent the total-groups Anostraca, Notostraca, and Diplostraca ([Laevicaudata + [Spinicaudata + [Cyclestherida + Cladocera]]]; see [41]), respectively [8–10], or at least stem Branchiopoda [42–44]. However, they differ in morphology and ecology from the Late Devonian Strud fossils and their modern representatives. Thus, the modifications that led to the long-lived ephemeral pool community evolved in a

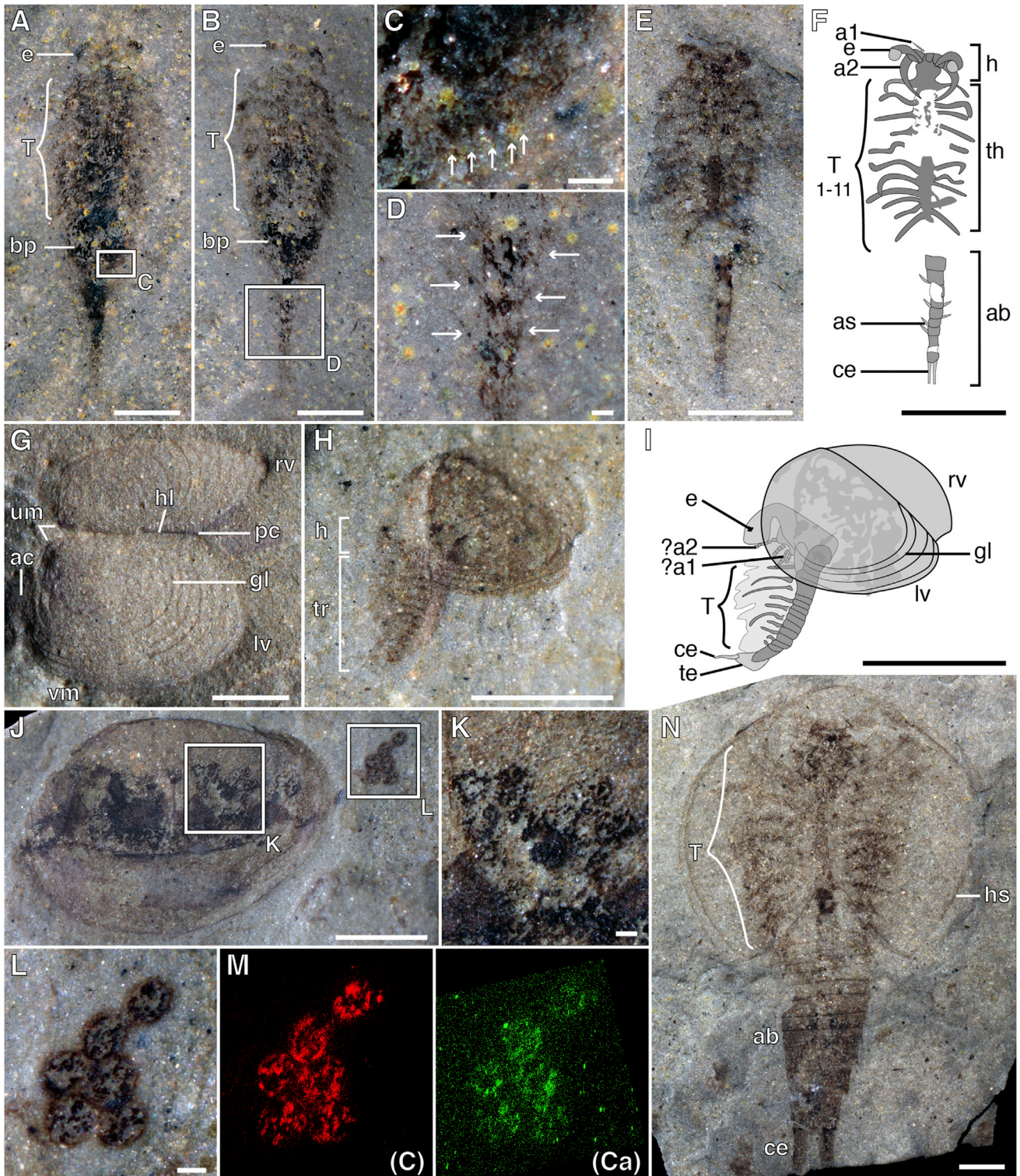


Figure 2. The Branchiopod Community from the Late Devonian of Strud, Belgium

(A–F) The anostracan *Haltinnaia serrata* gen. et sp. nov.

(A–D) Holotype, IRSNB a 12928a, b, female with a spiny abdomen and a unique, distally serrated circular brood pouch.

(A) IRSNB a 12928a.

(B) IRSNB a 12928b.

(C) IRSNB a 12928a, close-up of the distally serrated (arrows) egg pouch, from the boxed area in (A).

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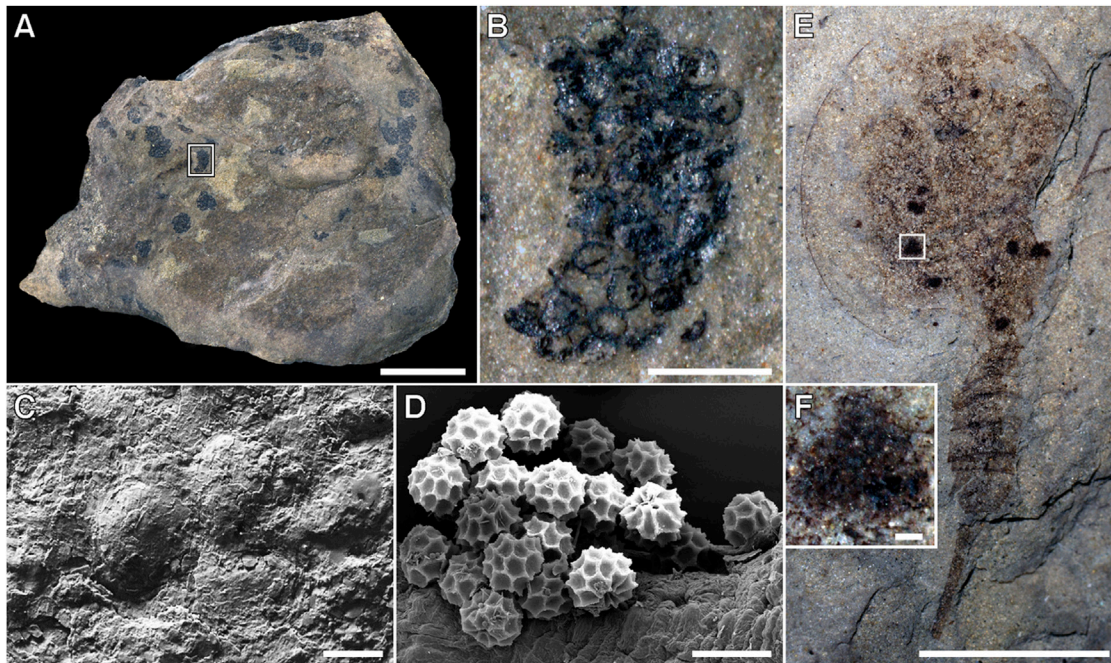


Figure 3. Egg Clusters within Strud Pool Deposits and the Notostracan *Strudops goldenbergi*

(A–C) IRSNB a 12937, branchiopod resting eggs.

(A) Branchiopod egg bank.

(B) Close-up of the egg cluster from the boxed area in (A).

(C) SEM image (back-scattered electron mode) showing three-dimensionally preserved resting eggs.

(D) SEM image (secondary electron mode) of an egg cluster from the extant spinicaudatan *Eulimnadia magdalenensis*.

(E and F) IRSNB a 12939, *Strudops goldenbergi* and resting eggs.

(E) Complete specimen with egg clusters between the limbs.

(F) Close-up of egg cluster from the boxed area in (E).

Scale bars represent 5 mm in (A) and (E), 500 μ m in (B), 100 μ m in (C) and (F), and 200 μ m in (D). See also [Figures S1E, S3, and S4](#).

different setting or sometime later, but prior to the Late Devonian.

Resting Eggs: A Key Innovation for Land Colonization

Terrestrial environments are characterized by fluctuating conditions and offer limited stability to potential colonizers. The ephemeral pool environment at least offers recurrent hydroperiods. The production of resting eggs, which accumulate as an egg bank in sediment and are resistant for long periods as they await favorable conditions [1, 2, 45], constitutes a reproductive strategy well suited to colonizing ephemeral pools and surviving seasonal desiccation. Particularly, the production of

thicker, more resistant egg membranes may have been a key innovation that favored the settlement of early branchiopods in ephemeral pools. Equally crucial was to acquire the ability to delay their egg development in response to adverse environmental conditions [46]. It is more parsimonious to suppose an ancestral acquisition of resting eggs in Branchiopoda in tandem with colonization of ephemeral pools [44], and long before the morphological specialization of each subclade, rather than convergent evolution of these attributes.

Together with the evolution of seeds in Devonian plants, the discovery of resting eggs in the Strud branchiopods provides rare fossil evidence of ecological adaptations to terrestrialization.

(D) IRSNB a 12928b, close-up of the abdomen bearing long spines (arrows), from the boxed area in (B).

(E and F) Paratype, IRSNB a 12930, male with more developed cephalic appendages.

(G–M) The spinicaudatan *Gesvesia pernegrei* gen. et sp. nov.

(G) Holotype, IRSNB a 12932, external morphology of the carapace.

(H and I) Paratype, IRSNB a 12934a, juvenile specimen.

(J) Paratype, IRSNB a 12936, internal anatomy showing resting eggs.

(K and L) IRSNB a 12936, close-ups of the egg clusters from the boxed areas in (J).

(M) SEM-EDX carbon and calcium elemental maps from (L).

(N) The notostracan *Strudops goldenbergi*. Paratype, IRSNB a 12859, complete specimen in dorsal view.

Abbreviations: a1, antennula; a2, antenna; ab, abdomen; ac, anterior corner; as, abdominal spines; bp, brood pouch; ce, cercopods; e, eye; gl, growth line; h, head; hl, hinge line; hs, head shield; lv, left valve; pc, posterior corner; rv, right valve; T, thoracopods; te, telson; th, thorax; tr, trunk; um, umbo; vm, ventral margin. *H. serrata* specimens are shown in dorsal view; *G. pernegrei* specimens are shown in lateral view with the head oriented to the left. Scale bars represent 1 mm in (A), (B), (E)–(J), and (N); 100 μ m in (C), (D), (K), and (L). See also [Figures S1 and S2](#).



Figure 4. Ecological Reconstruction of the Branchiopod Community from the Late Devonian of Strud, Belgium

Bottom left, the spinicaudatan *Gesvesia pernegrei*. Middle, the anostracan *Haltinnaia serrata*, male (bottom) and female (top). Top left and right, the notostracan *Strudops goldenbergi*. Eggs and egg clusters are laid on the bottom of the pool and accumulate in the sediment to constitute egg banks. Transverse section shows the successive desiccation events recorded in the sediment. Plant remains are fertile axes of the early seed plant *Moresnetia*. Illustration by Sophie Fernandez (MNHN).

The colonization of land started with the emergence of land plants in the Ordovician (circa 470 million years ago [mya]) [47, 48] and continued through the Late Silurian and Devonian (433.4–358.9 mya) with terrestrial arthropods [48–51] and tetrapods [52]. By the Middle to Late Devonian, terrestrialization generated new complex ecosystems on land, with diverse forest-like communities [53, 54], and irreversibly changed atmospheric $p\text{CO}_2$ and $p\text{O}_2$, climate, and sedimentation patterns [55–57]. The colonization of the ephemeral pool habitat by the branchiopod community during the Late Devonian, together with the presence of early tetrapods in surrounding flood channels [14], suggests that ephemeral pools and more generally freshwater bodies may have been key environments in the transition of animals from water to land.

Ecological Success and Evolution of the Branchiopod Ephemeral Pool Community

Besides the production of resting eggs, the success of large branchiopods in ephemeral pools may also reflect their various regimes as suspension feeders, grazers, or active predators: they do not depend on a single food source [1, 2, 46]. In addition, they hatch rapidly and have a relatively short life cycle with high fecundity, an essential condition to survive the ephemeral nature of the habitat [45]. Another important trait is their capacity to spread geographically to survive the isolation of ephemeral pools. Passive dispersion of resting eggs by wind during dry periods and occasionally by water (e.g., large flooding, temporary rivers) [1, 2, 45] allows branchiopods to colonize distant areas. This strategy appears to have been established at least by the Late Devonian. The size and shape of the eggs are significant because they influence transport by the wind [58]. Extant branchiopod eggs are also dispersed by endozoochory (ingestion by vertebrates such as birds and mammals), a directed passive dispersal vector that enhances the chance of reaching a suitable habitat (see [59]), but this would have been unlikely in the Devo-

nian because comparable vectors did not yet exist. The egg size of $163\ \mu\text{m}$ in the spinicaudatan *G. pernegrei* suggests that eggs stayed relatively constant in size in this group from the Late Devonian to the present, generally below $200\ \mu\text{m}$ [33, 60]. On the other hand, the $71\text{-}\mu\text{m}$ eggs of the notostracan *S. goldenbergi* are particularly small compared to those of extant examples, which are characterized by eggs more than $400\ \mu\text{m}$ in diameter [60]. The sparse fossil record of notostracan eggs shows variation in size through time, from $70\ \mu\text{m}$ in the Late Devonian to $150\text{--}160\ \mu\text{m}$ in the Upper Triassic of China [33] and $120\ \mu\text{m}$ and $400\ \mu\text{m}$ in the Lower Cretaceous of Mongolia [61] and China [62], respectively. This suggests a switch from dispersion by wind to endozoochory. The diversification of mammal and avian lineages in the Cretaceous may have played a major role in the dispersal success of the large-egged notostracans. Potential predators in recent ephemeral habitats also include aquatic insects, amphibians, and sometimes branchiopods themselves [1, 2]. Besides their important role in egg dispersal, these predators feed mainly on adult branchiopods.

The ecological and morphological stasis may be explained by the mixing of eggs from decades-distant populations, a singularity likely to prevent the fixation of new phenotypic variations [59]. Nonetheless, the apparent morphological stasis does not mean that these clades did not evolve through time, but rather that the changes are cryptic, as revealed by changes in egg size. In addition, variations in physiology and egg hatching phenology have been reported for several species without significant morphological change and seem to be important for the long-term occupation of ephemeral pool biotopes [11]. Fishes are generally absent in ephemeral pools, and increased fish predation in marine and fluvial environments during the Devonian may have triggered the modifications that allowed large branchiopods to colonize these continental environments devoid of predators [11]. Paradoxically, the variable and harsh ephemeral pool appears to have been one of the most stable continental ecosystems over hundreds of millions of years.

EXPERIMENTAL PROCEDURES

Fossil specimens were observed under a binocular microscope with polarized light both dry and covered in 95% ethanol. Drawings were produced using a

camera lucida, and photographs were taken under ethanol with a Canon EOS 5D Mark III camera coupled with a Canon MP-E 65-mm macro lens equipped with polarizing filters. Measurements were performed using ImageJ software. SEM images presented in Figures 3C and 3D were taken using Jeol benchtop SEM (JCM-6000) and Tescan SEM (VEGA II LSU), respectively. Elemental maps in Figure 2M were collected using the Tescan SEM associated with an X-ray detector type SD³ (Bruker).

ACCESSION NUMBERS

Fossil material described herein is housed at the Royal Belgian Institute of Natural Sciences, Brussels (IRSNB, Belgium): *Haltinnaias serrata*: IRSNB a 12928–12931; *Gesvesia pernegrei*: IRSNB a 12932–12936; egg banks: IRSNB a 12937–12938.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.12.039>.

AUTHOR CONTRIBUTIONS

P.G., N.R., and O.B. analyzed the fossils and prepared the figures. G.C. and O.B. designed the project. All authors contributed to the interpretation of results and drafted the manuscript.

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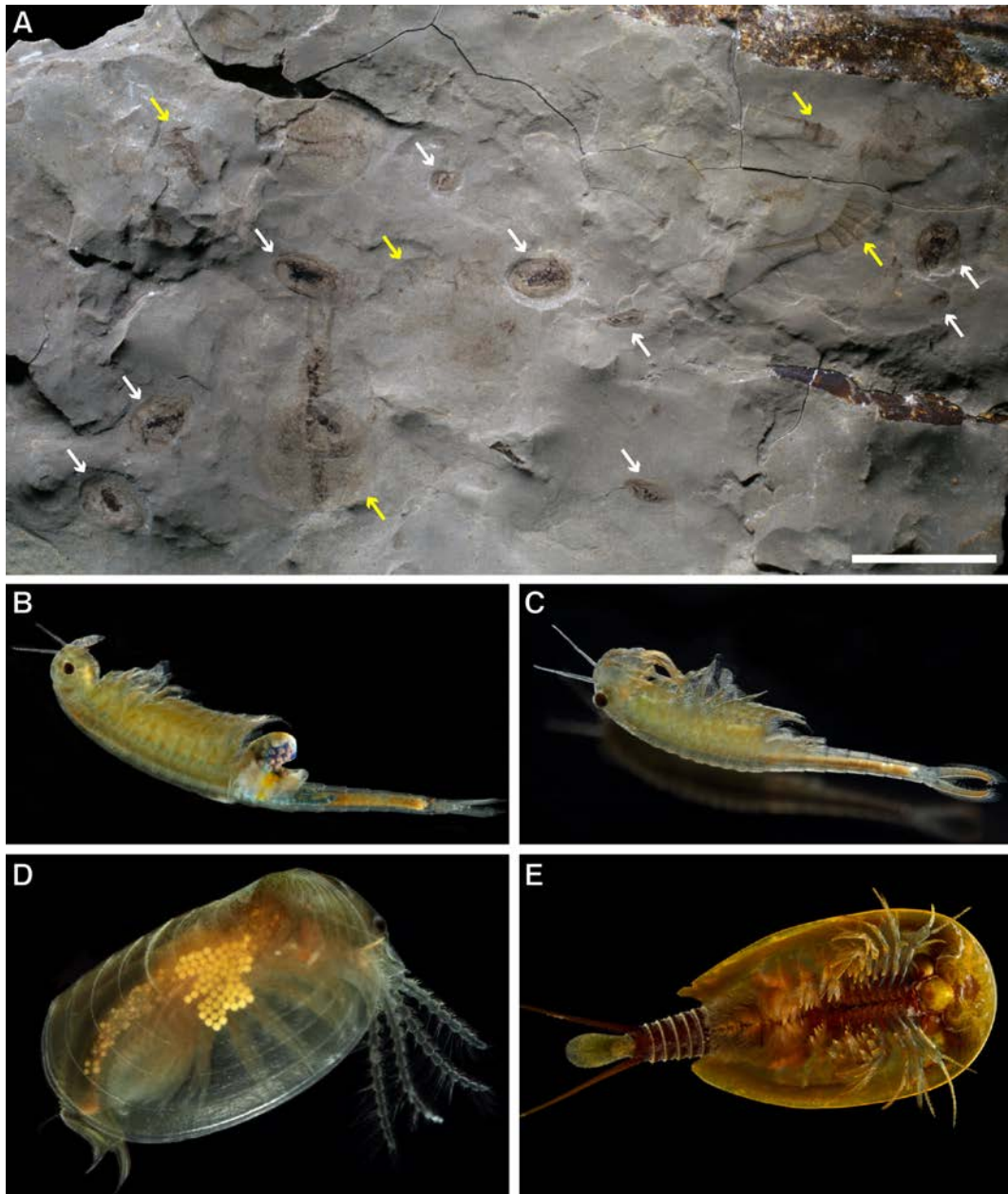


Figure S1. The branchiopod community from the Late Devonian of Strud, Belgium and examples of extant Anostraca, Spinicaudata and Notostraca. Related to Figures 2 and 3

(A) photograph (dry-ethanol composite) of a branchiopod-rich slab from the Strud locality, white and yellow arrows indicating spinicaudatan and notostracan remains respectively.

Scale bar represents 1 cm.

(B–E) examples of extant branchiopods, photos by Jean-François Cart.

(B) the anostracan *Branchipus schaefferi*, female with brood pouch holding resting eggs.

(C) the anostracan *Branchipus schaefferi*, male with antennae modified into large claspers to grasp the female during copulation.

(D) the spinicaudatan *Eoleptestheria ticinensis*, female bearing eggs.

(E) the notostracan *Lepidurus apus*, female bearing eggs.

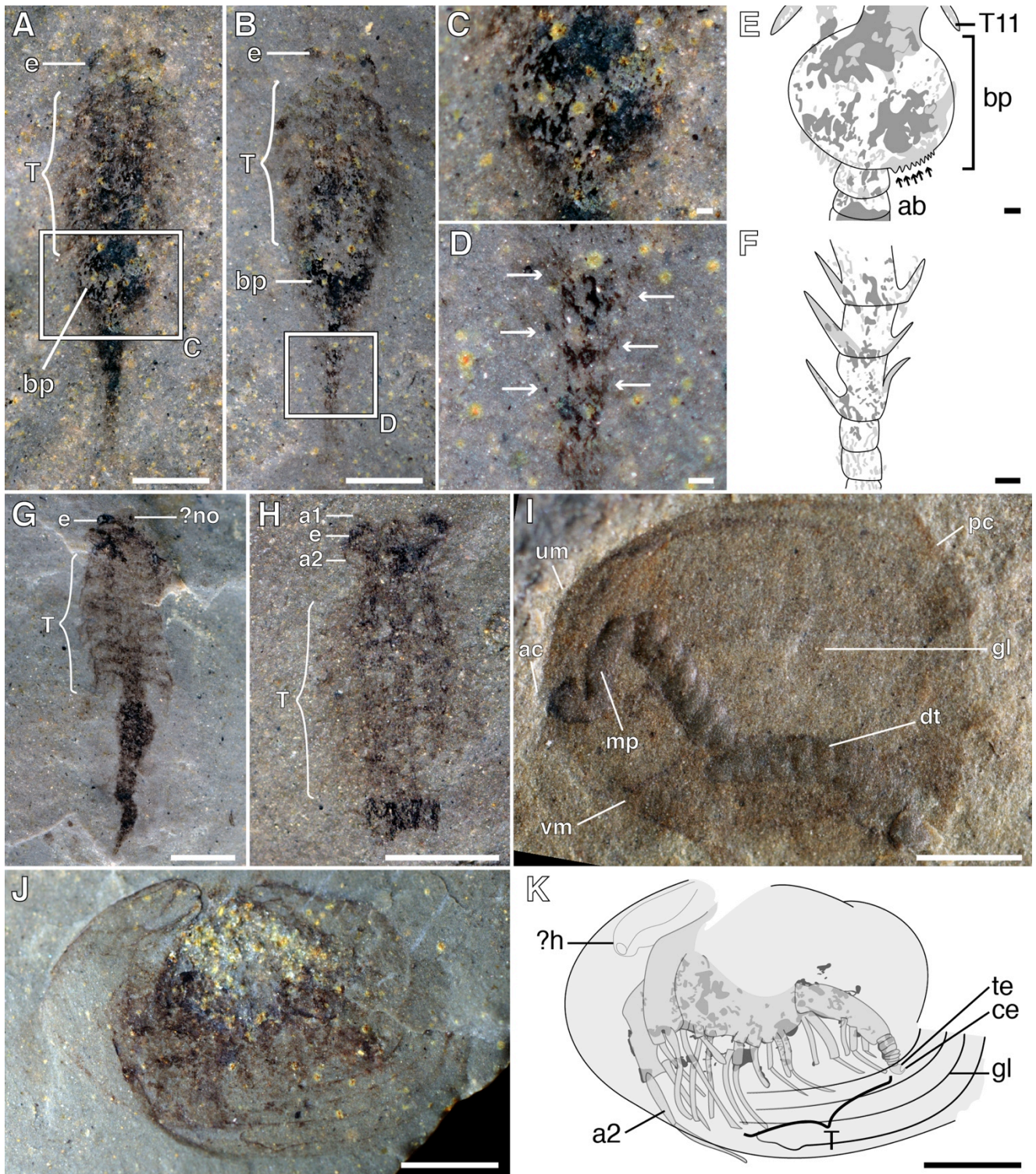


Figure S2. The anostracan *Haltinnaias serrata* gen. et sp. nov. and the spinicaudatan *Gesvesia pernegrei* gen. et sp. nov. from the Late Devonian of Strud, Belgium. Related to Figure 2

(A–F) *H. serrata* gen. et sp. nov., holotype, IRSNB a 12928a, b, female with a spiny abdomen and a unique, distally serrated, circular brood pouch. (A) IRSNB a 12928a. (B) IRSNB a 12928b. (C and E) IRSNB a 12928a, close-up of the distally serrated (arrows) egg pouch, from the box area in (A). (D

and F) IRSNB a 12928b, close-up of the abdomen bearing long spines highlighted by arrows, from the box area in (B).

(G) *H. serrata* gen. et sp. nov., paratype, IRSNB a 12929, specimen in dorsal view with thoracopods bearing setae and possibly a nuchal organ.

(H) *H. serrata* gen. et sp. nov., paratype, IRSNB a 12931a, most probably a male in dorsal view.

(I) *G. pernegrei* gen. et sp. nov., paratype, IRSNB a 12933, morphology of the digestive system.

(J and K) *G. pernegrei* gen. et sp. nov., paratype, IRSNB a 12935a, internal anatomy showing antenna, thoracopods and the abdomen ending in a conical telson with curved backward facing cercopod.

Abbreviations: a1, antennula; a2, antenna; ab, abdomen; ac, anterior corner; bp, brood pouch; ce, cercopod; dt, digestive tract with spiralled content; e, eye; gl, growth line; ?h, ?head; mp, mandibular molar process; ?no, ?nuchal organ; pc, posterior corner; T, thoracopods; te, telson; um, umbo; vm, ventral margin.

H. serrata specimens are in dorsal view; specimens of *G. pernegrei* are in lateral view, the head to the left.

Scale bars represent 1 mm in (A), (B) and (G)–(K) and (N); 100 μm in (C–F).

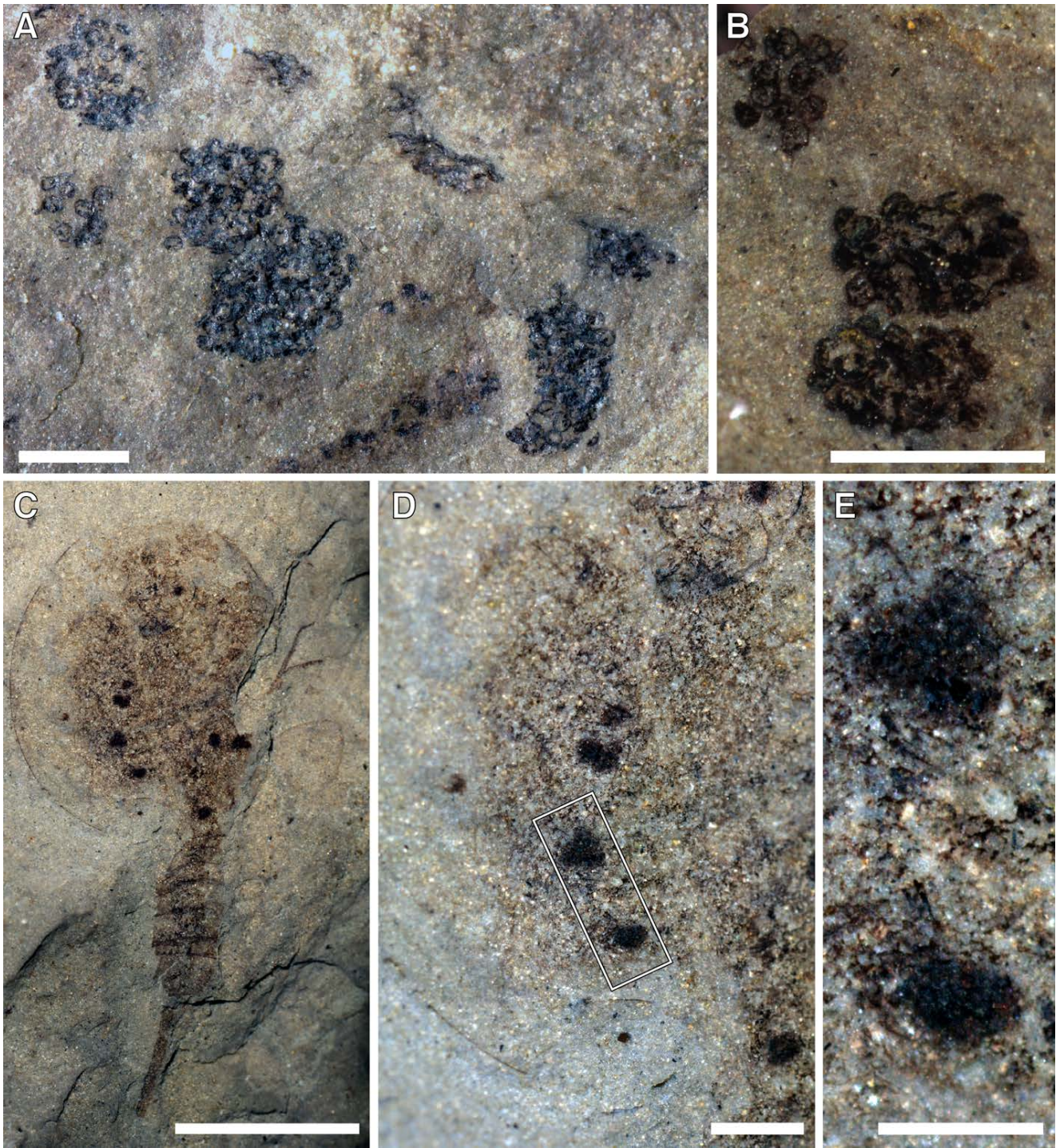


Figure S3. Branchiopod eggs that have been measured from clusters within Strud pool deposits and within the notostracan *Strudops goldenbergi*. Related to Figure 3

(A and B) egg banks IRSNB a 12937 and IRSNB a 12938.

(C–E) *S. goldenbergi*, IRSNB a 12939, egg clusters. (E) IRSNB a 12939, egg clusters that have been measured, from the white box area in (D).

Scale bars represent 1mm in (A), (B) and (D); 5 mm in (C); 500 μm in (E).

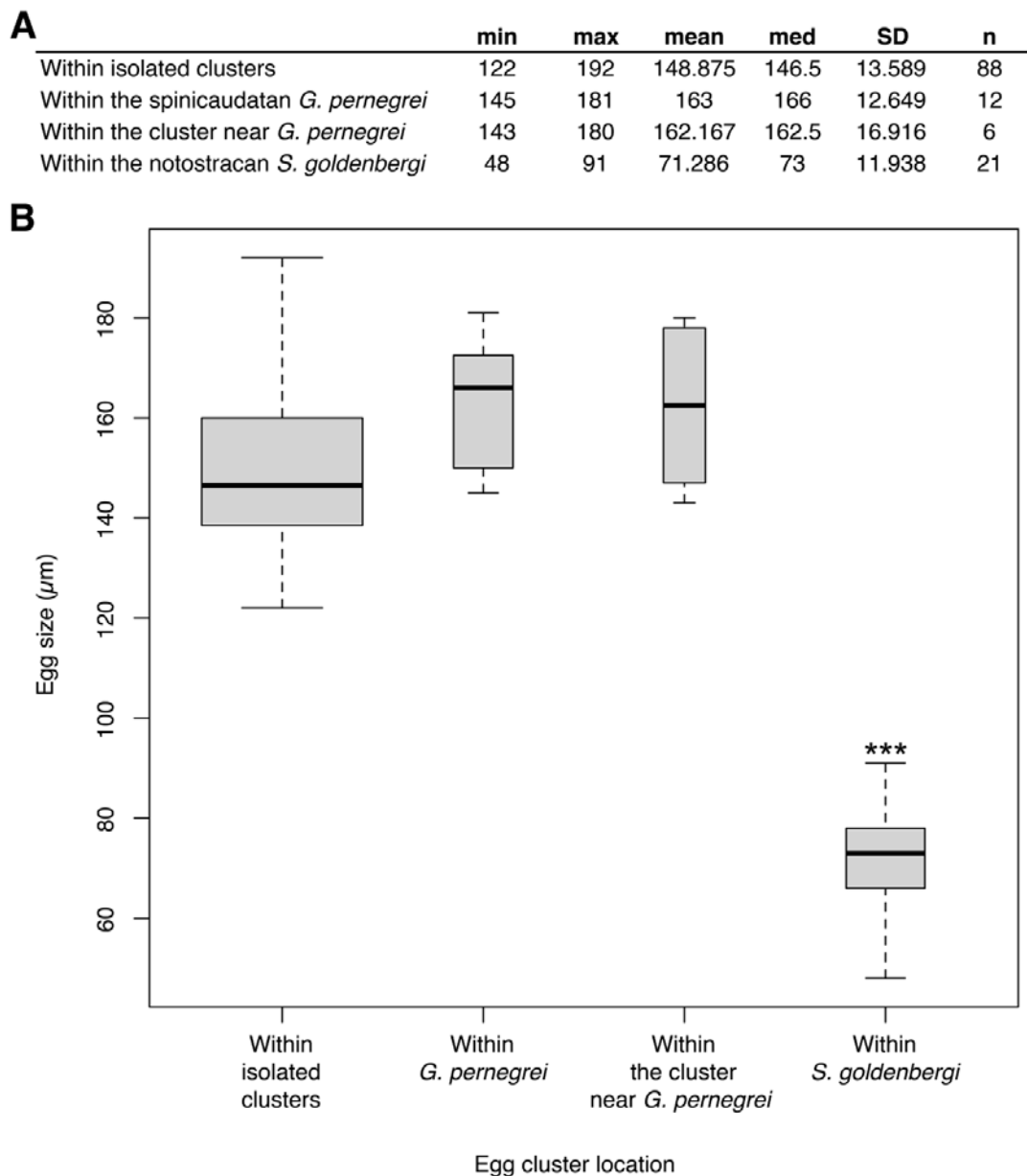


Figure S4. Egg size from clusters isolated in the Strud pool deposits and within branchiopods.

Related to Figure 3

(A) table indicating the minimum, maximum, mean and median sizes (μm) of eggs from isolated clusters in Strud pool deposits (IRSNB a 12937 and IRSNB a 12938, [Figures 3A, 3B, S3A and S3B](#)), within the spinicaudatan *Gesvesia pernegrei* (IRSNB a 12936, [Figures 2J and 2K](#)), near *G. pernegrei* (IRSNB a 12936, [Figure 2J and 2L](#)) and within the notostracan *Strudops goldenbergi* (IRSNB a 12939, [Figure 3E, 3F, and S3C–S3E](#)).

(B) corresponding boxplots showing a significant difference (highlighted by the stars) between egg size within *S. goldenbergi* and egg size within egg clusters found as egg banks within the pool deposits and within *G. pernegrei* (ANOVA p -value $< 2e-16$; a Tukey's Honestly Significant Difference test confirmed the egg size in *S. goldenbergi* to be different from that of the other eggs).