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Discontinuous gas-exchange in centipedes and its convergent evolution in tracheated arthropods

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Summary

We have examined the gas-exchange characteristics of five southern African centipede species from three orders. Two scolopendromorph species exhibit discontinuous gas-exchange cycles (DGCs) identical to those recorded for several insect and chelicerate species. Another scolopendromorph and a lithobiomorph species exhibit weak periodic patterns, and a scutigermorph species shows continuous gas exchange. A crucial component for DGCs in tracheated arthropods is the presence of occludible spiracles. However, on the basis of studies of temperate centipedes, most recent invertebrate biology texts hold the view that centipedes, as a group, cannot close their spiracles. Using flow-through normoxic and normoxic–anoxic–normoxic respirometry and electron microscopy, we conclusively demonstrate that at least one of the scolopendromorph species, *Cormocephalus morsitans* L., can close its spiracles fully, thus accounting for its DGCs. Homologies in spiracular structure and DGCs suggest that several other tracheated arthropod taxa probably have this ability too and that DGCs have evolved convergently at least four times in the Arthropoda. Spiracular closure and discontinuous gas-exchange cycles are probably more widespread in arthropods than has previously been suspected.

Key words: Chilopoda, Scolopendromorpha, centipede, *Cormocephalus morsitans*, spiracle, NAN respirometry, metabolic rate.

Introduction

Since the 1950s, discontinuous ventilation has been documented in many arthropods that possess tracheal systems and occludible spiracles. Levy and Schneiderman (1966) provided the first detailed descriptions of the discontinuous gas-exchange cycle (DGC) in lepidopteran pupae, and it has now been documented in insect species from several orders (Harrison, 1997; Lighton, 1998; Davis et al., 1999). The DGC has also been recorded in several other tracheated arthropod taxa including ticks (Lighton and Fielden, 1995; Lighton et al., 1993), soliphuges (Lighton and Fielden, 1996) and pseudoscorpions (Lighton, 1998). Typically, a DGC consists of closed (C), flutter (F) and open (O) phases. Transitions between these phases are regulated by both central-nervous-system-mediated and peripherally mediated endotracheal gas concentration set points (Lighton, 1994, 1996; Harrison, 1997). These set points are controlled by a central pattern generator (Hustert, 1975; Janiszewski and Otto, 1989; Ramirez and Pearson, 1989; Gulinson and Harrison, 1996; Bustami and Hustert, 2000).

Permeating much of the recent work on DGCs is the idea that these cycles are adaptive and have evolved in response to one or several specific environmental conditions (e.g. hypoxia, desiccation) (for reviews, see Kestler, 1985; Lighton, 1996, 1998), i.e. that natural selection has been responsible for both the origin and maintenance of either the entire DGC or its phase characteristics; for discussions of adaptation and natural selection, see Endler (1986) and Baum and Larson (1991). Several experimental investigations have tested one or more of the adaptive hypotheses proposed to account for the evolution of the DGC (e.g. Lighton and Berrigan, 1995; Chown and Holter, 2000). However, a comparative approach, which would indicate whether the DGC has arisen once or several times, thus providing grounds at least for a search for adaptive explanations (see Endler, 1986; Coddington, 1988; Baum and Larson, 1991; Brooks and McLennan, 1991), has not been adopted. Such an approach would be especially useful at the class level, within the Arthropoda, because fossil evidence indicates that invasion of terrestrial habitats occurred independently and at different geological periods in each of the major tracheated arthropod taxa (i.e. Insecta, Myriapoda, Chelicerata) (Bergstrom, 1979; Kukalova-Peck, 1991; Pritchard et al., 1993; Labandeira, 1999).

The first known terrestrial arthropods were probably chilopod-like myriapods dating back to the late Silurian (430 million years ago) (Robison, 1990; Johnson et al., 1994; Palmer, 1995). Earlier myriapods were marine, and the chelicerates and crustaceans also have numerous fossilised marine representatives, pre-dating the first terrestrial

Table 1. *Collection localities, mean annual rainfall, temperatures and body masses of the centipede species examined in this study*

Species	Locality	Grid reference	Mean annual rainfall (mm)	Annual temperature (°C)			Body mass (g)			
				Mean	Minimum	Maximum	Mean	Minimum	Maximum	*N*
Cormocephalus morsitans	Pietersburg	23.87 °S, 29.45 °E	458	22.8	17.1	28.5	1.6122±0.4059	0.2886	3.7016	9
C. brevicornis	Pietersburg	23.87 °S, 29.45 °E	458	22.8	17.1	28.5	0.0772±0.0146	0.0479	0.1285	5
C. elegans	Pretoria and	25.75 °S, 28.17 °E	652	22.5	16.5	28.6	1.1747±0.2460	0.0386	1.9252	10
	Mooketsi	25.58 °S, 30.08 °E	594	24.1	18.7	29.6				
Scutigerina weberi	Pretoria and	25.75 °S, 28.17 °E	652	22.5	16.5	28.6	0.1055±0.0357	0.0079	0.2300	5
	Mooketsi	25.58 °S, 30.08 °E	594	24.1	18.7	29.6				
Lithobius melanops	Gough Island	40.33 °S, 10.0 °E	2445	14.0	11.1	16.9	0.0212±0.0021	0.0109	0.0292	10

Climate data were extracted from IPCC (Intergovernmental Panel on Climate Change) Data Distribution Centre (http://ipcc-ddc.cru.uea.ac.uk/ipcc_ddc/cru_data/datadownload/download_index.html).

Values for body mass are means ± S.E.M.

myriapods, although the first known chelicerate and crustacean terrestrial representatives are younger than the first terrestrial myriapods. The insects as a group appear to have evolved exclusively on land, with archaeognathan representatives appearing as early as the Devonian (390 million years ago), although recognisably herbivorous insects only appeared in the Carboniferous (Bergstrom, 1979; Kukalova-Peck, 1991; Pritchard et al., 1993). Therefore, if DGCs were found in all these taxa, there would be good grounds for suggesting that the transition to terrestriality always leads to the evolution of DGCs and that DGCs therefore provide some adaptive advantage to terrestrial, tracheated arthropod species.

To date, DGCs have been recorded in the Chelicerata (Lighton et al., 1993; Lighton and Fielden, 1996) and the Insecta (Lighton, 1994, 1996, 1998). However, there is little information on gas exchange in myriapods, and particularly not for the Chilopoda. Since the late 1880s, it has been known that centipedes show a remarkable diversity in spiracle structure, with at least some species, especially those in the Scolopendromorpha, possessing a morphology and anatomy that indicate an ability to close their spiracles completely (Lewis, 1981; Lewis et al., 1996). Indeed, Lewis (1981 and Lewis et al., 1996) argued that many features of centipede spiracles (irrespective of whether they can close or not) might have evolved to combat water loss (but see Curry, 1974), thus echoing similar claims made for insects and other arthropods (e.g. Kestler, 1985; Pugh, 1997; Lighton, 1998). Nonetheless, there have been remarkably few investigations of respiratory metabolism in centipedes (but see Crawford et al., 1975; Riddle, 1975) and none of the gas-exchange characteristics of these arthropods.

In this paper, we therefore examine the distribution of discontinuous gas-exchange cycles across the major classes of tracheated arthropods. We do so by examining the existing data in a phylogenetic context and by adding information on five species of centipede (Chilopoda) from three orders, Scolopendromorpha (three species), Lithobiomorpha (one species), and Scutigeromorpha (one species), and a variety of habitats. Our aims are severalfold. First, we determine whether there is any evidence that centipede species can close their spiracles, contrary to widely held modern opinion (see Curry, 1974; Little, 1990; Withers, 1992; Ruppert and Barnes, 1994), and whether any variation in this ability among species is reflected in spiracle structure. Second, we characterise gas-exchange patterns in these species. Finally, and using information both from this study and from the literature, we revisit the question of the origin of the DGC in arthropods. In doing so, we follow the lead of Lighton (1996, 1998), who has not only pressed for the documentation and investigation of the DGC in as wide an array of taxa as possible but also encouraged investigators to acknowledge the variability of the DGC and to publish those instances in which it is simply not present (so overcoming the 'file drawer problem') (see Csada et al., 1996).

Materials and methods

Study animals

Three centipede species in the Order Scolopendromorpha were examined. *Cormocephalus morsitans* Linnaeus and *Cormocephalus brevicornis* Kraepelin (Class: Chilopoda, Order: Scolopendromorpha) were both collected from semi-arid savanna in southern Africa (see Table 1 for localities and climate information), while the third species in this genus, *C. elegans* Kraepelin, was collected in more mesic habitats from the University of Pretoria Botanical Gardens and the Mooketsi Valley. *Lithobius melanops* (Lithobiomorpha), a cosmopolitan species, was collected from mid-Atlantic Gough Island, where it lives in very moist fernbush forests, and *Scutigerina weberi* Silvestri (Scutigeromorpha) was collected from mesic habitats in Pretoria and the Mooketsi Valley.

Respirometry

Following collection, individuals were kept in the laboratory

in climate chambers regulated at 20±1 °C with a 12 h:12 h L:D photoperiod. Prior to investigation, individual centipedes were starved for at least 24 h on moist soil. An individual was then weighed (to 0.01 mg, on a Sartorius Research electronic microbalance) and placed in a cuvette located in a darkened water jacket connected to a Grant LTD20 water bath, which maintained temperature at 20±0.2 °C. The individual was allowed to settle for 60 min, after which respirometry commenced. A Sable Systems flow-through CO_2 respirometry system (Sable Systems, Henderson, Nevada, USA) was used to investigate gas-exchange characteristics. Synthetic air (21 % O_2, balance N_2) was passed through sodalime, silica gel and Drierite columns to remove CO_2 and H_2O residues. From there, the clean air flowed at a steady rate (see below) through an automatic baselining system, the cuvette and then a LiCor 6262 CO_2/H_2O infrared gas analyzer. The LiCor gas analyzer and other Sable Systems peripheral equipment were connected to a desktop computer using Datacan V software for data capture and control of the respirometry system.

Fifteen minutes into the settling period, a baseline measurement was made by bypassing the cuvette. The centipede was then allowed to equilibrate to flowing air for 45 min, after which respirometry measurements commenced. Depending on the size of the centipede, cuvettes with a volume of either 5 cm^3 or 60 cm^3 were used (gas flow rates were adjusted accordingly to 50 or 200 ml min^{-1}, respectively). Measurements were made for 3–18 h, depending on centipede size (see Chown, 2001). To prevent severe desiccation in the more mesic centipede species (all species except *C. morsitans*), CO_2- and H_2O-free air was rehumidified (to a vapour pressure of 1.704 kPa at 20 °C) by inserting a LiCor 610 dewpoint generator between the automatic baselining system and the cuvette. CO_2 contamination of the air from the LiCor dewpoint generator was prevented by inserting a second sodalime scrubber column between the dewpoint generator air outlet and the cuvette inlet. *Cormocephalus morsitans* specimens were examined using dry and moistened air. All measurements were corrected to standard temperature and pressure and expressed as ml CO_2 h^{-1}.

NAN respirometry

NAN (normoxic–anoxic–normoxic) respirometry (Lighton and Fielden, 1996) was used to determine *in vivo* whether centipedes that seemed to have the ability to close their spiracles could actually do so. The rationale for this test, which involves replacing normoxic air with pure nitrogen following closure of the spiracles, is as follows. If the spiracles are effectively closed, the anoxic air should have no influence on the endotracheal P_{O_2} or on the gas exchange of the animal. In insects, with the decline in endotracheal P_{O_2}, the spiracles normally open as a result of a centrally mediated P_{O_2} set point of approximately 5 kPa, and this corresponds to the flutter phase initiated by the low endotracheal P_{O_2} (Lighton, 1994, 1996). Anoxic air would, however, prevent the inward diffusion of oxygen. Indeed, diffusion outwards should result in a rapid loss of endotracheal oxygen, causing complete opening of the spiracles and a large burst emission of CO_2. Resupplying the animals with normoxic air at the end of the CO_2 burst should allow the animal to recover fully and should be demonstrated by the resumption of the normal DGC starting with a closed phase. If this sequence of events were to take place, it would be strong evidence for a gas-exchange cycle equivalent to the DGC found in insects (Lighton and Fielden, 1996).

In this instance, individual centipedes that had gas-exchange characteristics indicative of complete spiracular closure were supplied with normoxic air (21 % O_2, balance N_2) until the CO_2 emission rates were very low. Normoxic air was then replaced with anoxic, pure nitrogen scrubbed of all CO_2 and H_2O residues. The experiments were undertaken at 15 °C to increase the duration of the closed phases during DGC in smaller specimens, which improves the resolution of the NAN investigations.

Spiracle configuration and structure

The number of body segments and the distribution and position of spiracles along these segments for each of the three higher taxa were noted, and the spiracles were examined using light microscopy. Large specimens of the scolopendromorph species that showed pronounced differences in gas-exchange characteristics (i.e. *C. elegans* and *C. morsitans*) were fixed in 100 % ethanol. Spiracle-bearing segments were dissected, and both longitudinal and transverse sections were made. The sectioned material was cleaned in an ultrasonic bath, dried in CO_2 in a critical point dryer, mounted on aluminium stubs, gold-coated in a Polaron sputter coater and examined and photographed using a JEOL 840 scanning electron microscope.

Analyses

Datacan V (Sable Systems, Henderson, Nevada, USA) was used for data capture and analyses of CO_2 emissions. Analyses of variance (ANOVAs) and covariance (ANCOVAs) (with body mass as covariant) were used for interspecific comparisons of metabolic rates and DGC parameters. Least-squares linear regressions of $\log_{10}$-transformed values were used to investigate allometric scaling of metabolic rates and DGC parameters.

Significance was set at P=0.05 throughout.

Results

Gas-exchange characteristics

The gas-exchange characteristics of the centipedes examined here showed a great deal of variation, between orders, within the genus *Cormocephalus* and within species (Tables 2, 3), the latter resulting mostly from substantial size variation among the specimens collected. The scutigerimorph *Scutigerina weberi*, which has tracheal lungs, appears to exchange gases continuously because no evidence of discontinuous gas exchange was found in the nine recordings made (Fig. 1E). In the other two mesic species, *C. elegans* (seven specimens, 18 recordings and 30 gas-exchange cycles

Table 2. *Mean CO_2 emission volumes (μl), phase durations (min) and gas-exchange coefficients of the centipede species examined in this study that showed recognisable cyclic gas-exchange patterns*

Species	C-phase	F-phase Interburst*	O-phase Burst*	Total	*N*	Mass (g)
Emission volumes (μl)						
Cormocephalus morsitans (dry)	4.401±1.653	36.039±14.738	59.726±18.161	100.167±33.730	9	1.863±0.53
C. morsitans (wet)	1.750±1.185	9.257±5.889	32.938±19.252	43.944±26.318	5	1.16±0.65
C. brevicornis	0.0418±0.007	0.548±0.052	0.984±0.191	1.566±0.248	5	0.0772±0.0146
C. elegans		92.895±36.573*	124.56±22.717*	217.351±48.105	7	1.17±0.25
Lithobius melanops		2.474±1.359*	1.401±0.454*	3.875±1.812	2	0.0212±0.0021
Scutigerina weberi	No cyclic respiratory patterns observed					
Phase duration (min) and gas-exchange coefficient (in parentheses)						
C. morsitans (dry)	20.89±5.81 (0.149)	113.35±38.86 (0.701)	11.33±1.11 (0.150)	145.57±45.36	9	
C. morsitans (wet)	11.86±3.02 (0.177)	41.12±7.96 (0.647)	9.77±1.07 (0.176)	62.75±11.43	5	
C. brevicornis	2.85±0.81 (0.071)	25.26±3.95 (0.742)	5.98±0.32 (0.187)	33.52±3.99	5	
C. elegans		80.10±16.36* (0.632)	41.99±5.95* (0.368)	122.09±20.38	7	
L. melanops		38.13±10.56* (0.667)	18.29±2.05* (0.333)	56.42±12.61	2	

Wet indicates rehumidified air, dry indicates dry air; see text for details.

DGC=C-phase + F-phase + O-phase, where DGC is discontinuous gas exchange, C is the closed phase, F is the flutter phase and O is the open phase.

*Characterizes the coefficients of those species that do not show DGCs but do show some form of cyclic gas exchange.

Values are means ± S.E.M.

analysed) and *L. melanops* (10 specimens, 10 recordings and six gas-exchange cycles analysed), which both have well-developed tracheal systems, a cyclic form of CO_2 emission, atypical of conventional DGCs, was found (Fig. 1C,D).

DGC patterns that are functionally indistinguishable from those typical of many insects were found in the two centipede species from xeric habitats, *C. morsitans* (nine specimens, 23 recordings and 106 DGCs analysed) and *C. brevicornis* (five specimens, six recordings and 29 DGCs analysed). Both species displayed DGCs with distinct closed (C), flutter (F) and open (O) phases (Fig. 1A,B), suggesting that these species are able to close their spiracles. NAN respirometry confirmed that *C. morsitans* close their spiracles completely during the 'closed' portion of the interburst phase (four specimens, seven recordings and nine cycles analysed). Measurements at 15 °C increased the duration of this closed phase to approximately 10 min (Table 4). When fluttering was initiated at the end of the closed phase, the anoxic atmosphere appeared to cause rapid depletion of the remaining endotracheal oxygen. The result was a complete opening of the spiracles and the emission of a large volume of CO_2. Resupply of normoxic air appeared to normalize the endotracheal oxygen levels, because a typical DGC resumed (Fig. 2). Summary statistics for emission volumes and durations confirmed the effect of anoxic air on the DGC (Table 4). Unfortunately, NAN respirometry was not undertaken on *C. brevicornis* because of the high mortality of this species in dry air, probably a consequence of their small size. Nonetheless, the pronounced DGC found in this species suggests that it is also able to close its spiracles.

Gas-exchange phase coefficients (*sensu* Davis et al., 1999) indicated that in both *C. morsitans* and *C. brevicornis* the DGC is dominated by the F-phase, with the C- and O-phases making equal, though smaller, contributions (Table 2). In *C. elegans* and *L. melanops*, the burst phase (equivalent to the O-phase in true DGCs) contributes one-third to the gas-exchange cycle. An ANCOVA (with body mass as covariate) indicated that the rates of CO_2 emission in the interburst phases of *C. elegans* and *L. melanops* are much higher than the rates of emission in the closed phases of *C. morsitans* and *C. brevicornis* ($F_{1,24}$=18.15, P<0.0003), suggesting substantial leakage of CO_2 from the spiracles of *C. elegans* and *L. melanops* (see also Fig. 1C,D).

CO_2 emission volumes and rates and phase durations all scaled positively and significantly with mass (Table 3). However, marked differences in the scaling exponents of CO_2 emission volumes and the rate of emission of CO_2 ($\dot{V}_{CO_2}$) meant that DGC frequency was inversely related to body size (Table 3). When converted to μW (Table 5) (assuming a respiratory quotient, RQ, of 0.6) (see Riddle, 1975), the scaling relationship for standard metabolic rate (SMR) was $SMR=331M^{0.630}$, where M is body mass. Assuming a more realistic RQ of 0.84 (Withers, 1992) gave a relationship of $SMR=257M^{0.630}$. When the two species showing DGCs were excluded from the scaling analysis because their

Table 3. *Results of least-squares linear regression analyses of respirometry variables, on body mass, of the phases comprising the gas-exchange cycles*

	Regression statistics					
DGC parameters	Slope	Intercept	r^2	$F_{d.f.}$	*N*	*P*
$\log_{10}$ (CO_2 emission volume)						
Closed (all true DGC species)	1.398±0.125	–7.152±0.353	0.89	$124.3_{1,16}$	18	<0.0001
Flutter (all species)	0.967± 0.151	–4.806±0.418	0.62	$42.6_{1,26}$	28	<0.0001
Flutter (all true DGC species)	1.190±0.087	–5.611±0.242	0.92	$186.2_{1,17}$	19	<0.0001
Interburst (all non-DGC species)	0.903±0.097	–3.863±0.262	0.94	$87.5_{1,6}$	8	<0.0001
Open (all species)	1.103±0.094	–4.723±0.260	0.84	$137.6_{1,26}$	28	<0.0001
Open (all true DGC species)	1.226±0.029	–5.250±0.080	0.99	$1794.8_{1,17}$	19	<0.0001
Open (all non-DGC species)	0.962±0.116	–3.970±0.314	0.92	$69.2_{1,6}$	8	<0.0002
$\log_{10}$ (DGC phase duration)						
Closed (all true DGC species)	0.690±0.089	–2.749±0.250	0.79	$60.4_{1,16}$	18	<0.0001
Flutter (all species)	0.328±0.081	–0.977±0.225	0.38	$16.2_{1,26}$	28	<0.0005
Flutter (all true DGC species)	0.473±0.097	–1.419±0.270	0.58	$23.6_{1,17}$	19	<0.0002
Interburst (all non-DGC species)	0.210±0.058	–0.479±0.156	0.69	$13.4_{1,6}$	8	<0.011
Open (all species)	0.178±0.082	–1.122±0.227	0.15	$4.7_{1,26}$	28	<0.04
Open (all true DGC species)	0.218±0.020	–1.412±0.054	0.88	$122.6_{1,17}$	19	<0.0001
Open (all non-DGC species)	0.208±0.035	–0.751±0.095	0.85	$35.1_{1,6}$	8	<0.001
$\log_{10}$ (CO_2 emission rate)						
Closed (all true DGC species)	0.709±0.083	–4.404±0.236	0.82	$71.8_{1,16}$	18	<0.0001
Flutter (all species)	0.659±0.101	–3.830±0.279	0.62	$42.7_{1,26}$	28	<0.0001
Flutter (all true DGC species)	0.717±0.038	–4.192±0.106	0.95	$347.7_{1,17}$	19	<0.0001
Interburst (all non-DGC species)	0.210±0.058	–0.479±0.156	0.69	$13.4_{1,6}$	8	<0.011
Open (all species)	0.926±0.060	–3.601±0.165	0.90	$240.2_{1,26}$	28	<0.0001
Open (all true DGC species)	1.009±0.036	–3.838±0.100	0.98	$785.8_{1,17}$	19	<0.0001
Open (all non-DGC species)	0.754±0.091	–3.219±0.248	0.92	$67.9_{1,6}$	8	<0.0002
$\log_{10}$ (gas-exchange frequency)						
All species	–0.377±0.081	0.398±0.224	0.45	$21.6_{1,26}$	28	<0.0001
All true DGC frequencies	–0.526±0.107	0.885±0.297	0.59	$24.2_{1,17}$	19	<0.0002
All non-DGC frequencies	–0.219±0.027	–0.222±0.074	0.91	$63.7_{1,6}$	8	<0.0003

DGC, discontinuous gas exchange.
CO_2 emission volume is in ml; DGG duration is in h; CO_2 emission rate is in ml h^{-1}; gas-exchange frequency is in mHz.
Values are means ± S.E.M.

SMRs appeared to be very variable (Table 5), the scaling relationships for metabolic rate were $SMR=575M^{0.676}$ and $SMR=439M^{0.676}$, with RQs of 0.6 and 0.84, respectively.

Spiracle configuration and structure

The scolopendromorph centipedes all have 21 body segments, each bearing one pair of uniramous legs. Nine pairs of spiracles are situated on leg-bearing segments 3, 5, 8, 10, 12, 14, 16, 18 and 20. *Lithobius melanops* has 15 body segments with a pair of spiracles on leg-bearing segments 3, 5, 8, 10, 12 and 14. From the spiracular openings, the tracheae innervate the surrounding organs in a way analogous to that in insects, forming tracheal interconnections between the spiracles (see also Lewis, 1981). *Scutigera weberi* has 15 leg-bearing body segments covered by eight sclerotized dorsal plates. On the middle of the posterior edge of each of these plates there is a single spiracular opening forming a longitudinal slit. Tracheae fan out left and right from these single slit-like spiracles to form tracheal lungs (see also Lewis, 1981).

Cormocephalus elegans, which shows no evidence of a DGC, has its spiracles situated directly above the leg. The spiracles of a 96 mm long *C. elegans* specimen had a slight triangular-shaped ostium (*sensu* Curry, 1974), which was 500 μm long (in longitudinal section), with the posterior portion being 250 μm wide (Fig. 3A). The ostium is lined with trichomes 10–30 μm long, and this lining extends approximately 100 μm into the sub-ostial space, where a bare and narrow (15–20 μm) cuticular fold separates the ostium from the tracheal atrium. The tracheal atria are densely lined with long atrial trichomes (50–100 μm) that completely cover all tracheal openings (Fig. 3B).

In *C. morsitans*, spiracular morphology is quite different. In the 65 mm long specimen photographed, the spiracles were situated dorsally but behind the posterior edge of the coxae. The ostial opening is also triangular, 300 μm long, and 130 μm wide on the posterior side. The first 30 μm of the ostium is lined with ostial trichomes 10–15 μm long. On the inner edge of the ostium, several ostial trichomes are elongated up to 50 μm. These longer

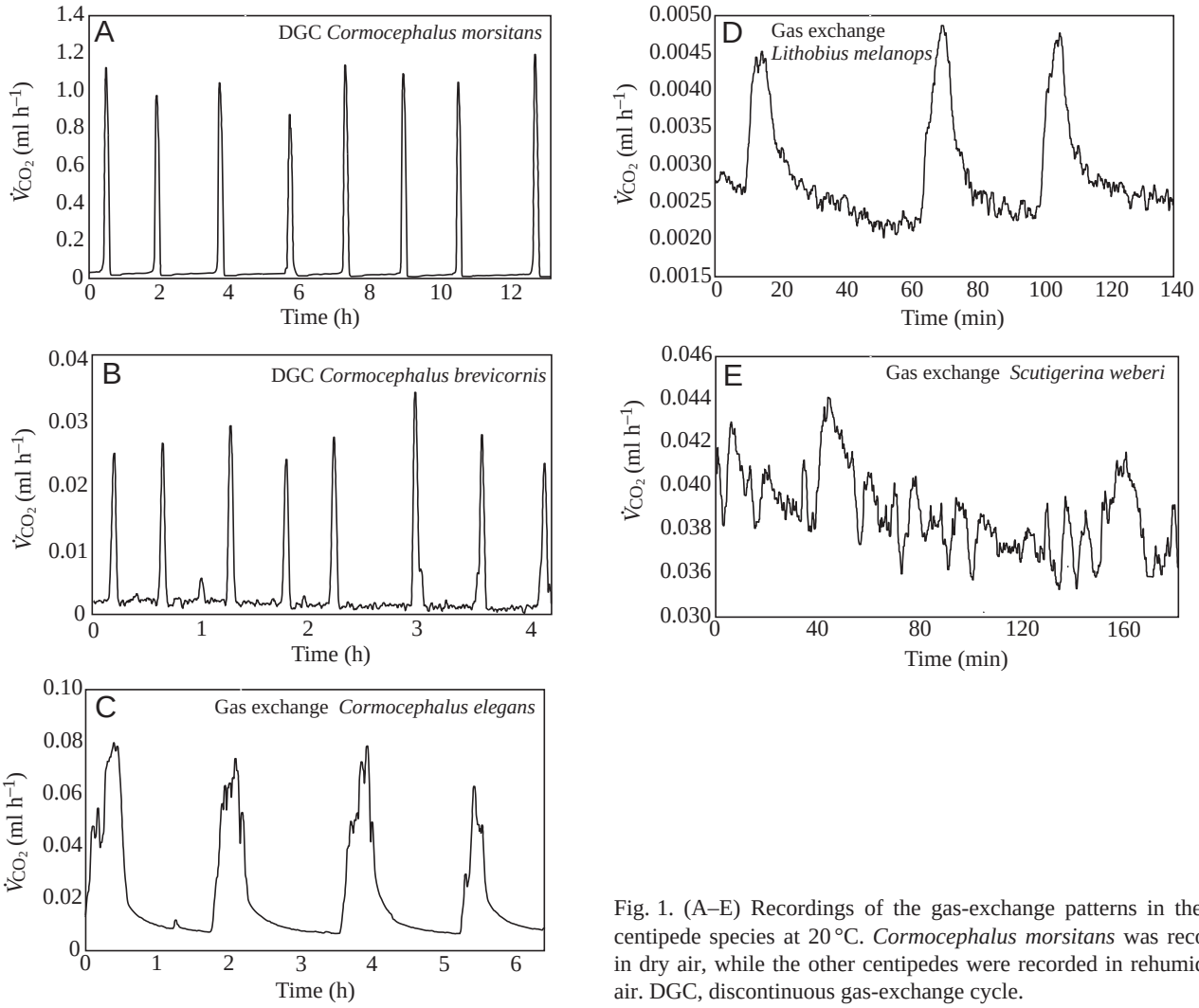

Fig. 1. (A–E) Recordings of the gas-exchange patterns in the five centipede species at 20 °C. *Cormocephalus morsitans* was recorded in dry air, while the other centipedes were recorded in rehumidified air. DGC, discontinuous gas-exchange cycle.

Table 4. *Mean gas-exchange phase durations (min) and CO_2 emission volumes (μl) determined with NAN respirometry for* Cormocephalus moristans *at 15 and 20 °C*

		C-phase	F-phase	O-phase
Normoxic–anoxic–normoxic (NAN) respirometry at 15 °C				
Normal DGC	Phase duration	10.62±5.00	38.06±7.50	11.69±0.54
	Emission volume	0.553±0.226	4.000±0.653	20.895±3.323
NAN respirometry	Phase duration	8.00±1.10	–	4.76±0.64
	Emission volume	0.562±0.154	–	12.137±1.358
Post-NAN DGC	Phase duration	6.17±1.07	66.48±6.26	12.38±1.86
	Emission volume	0.222±0.038	5.647±1.028	22.309±5.229
NAN respirometry at 20 °C				
Normal DGC	Phase duration	3.95±2.88	20.11±3.79	8.35±1.25
	Emission volume	0.182±0.122	3.159±0.551	18.528±4.066
NAN respirometry	Phase duration	1.79±1.228	–	5.157±0.74
	Emission volume	0.092±0.040	–	11.748±1.751
Post-NAN DGC	Phase duration	1.76±1.11	20.66±2.55	8.6±1.02
	Emission volume	0.067±0.030	3.061±0.549	11.366±5.191

Values are means ± S.E.M. (N=3).
DGC, discontinuous gas exchange; C, closed phase; F, flutter phase; O, open phase.

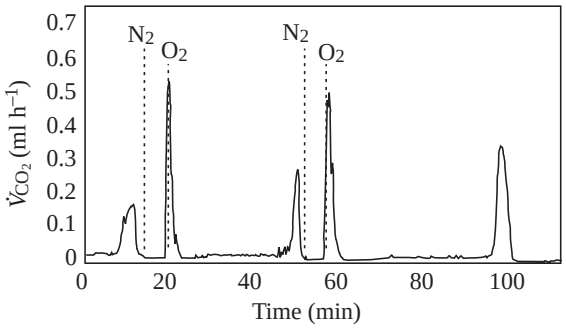

Fig. 2. A typical normoxic–anoxic–normoxic respirometry recording for *Cormocephalus morsitans* at 20 °C in dry air. The markers indicate when the airflow was changed from normoxic (21 % O_2, balance N_2) to anoxic (pure N_2) and back to normoxic again. The large bursts of CO_2 emission indicate the end of the closed phase, when the centipede initiates a flutter phase (functionally equivalent to those observed in insects) to maintain O_2 partial pressure sufficient for cellular respiration.

Table 5. *Standard metabolic rates of the five centipede species*

	Mean SMR (μW)		
Species	RQ=0.6	RQ=0.84	*N*
Cormocephalus morsitans	312.28±57.24^{b}	237.55±43.54^{b}	14
C. brevicornis	25.91±3.64^{c}	19.71±2.77^{c}	5
C. elegans	623.89±128.14^{a}	474.60±97.48^{a}	10
Lithobius melanops	40.70±5.56^{c}	30.96±4.23^{c}	10
Scutigerina weberi	135.86±45.41^{b,c}	103.35±34.54^{b,c}	5

ANCOVA of SMR between species; body mass as covariant
Assuming an RQ of 0.6, see Riddle (1975) $F_{4,38}$=8.92, P<0.0001
Assuming an RQ of 0.84, see Withers (1992) $F_{4,38}$=8.92, P<0.0001

Values are means ± S.E.M.
RQ, respiratory quotient; SMR, standard metabolic rate.
Different superscripts denote significant differences.

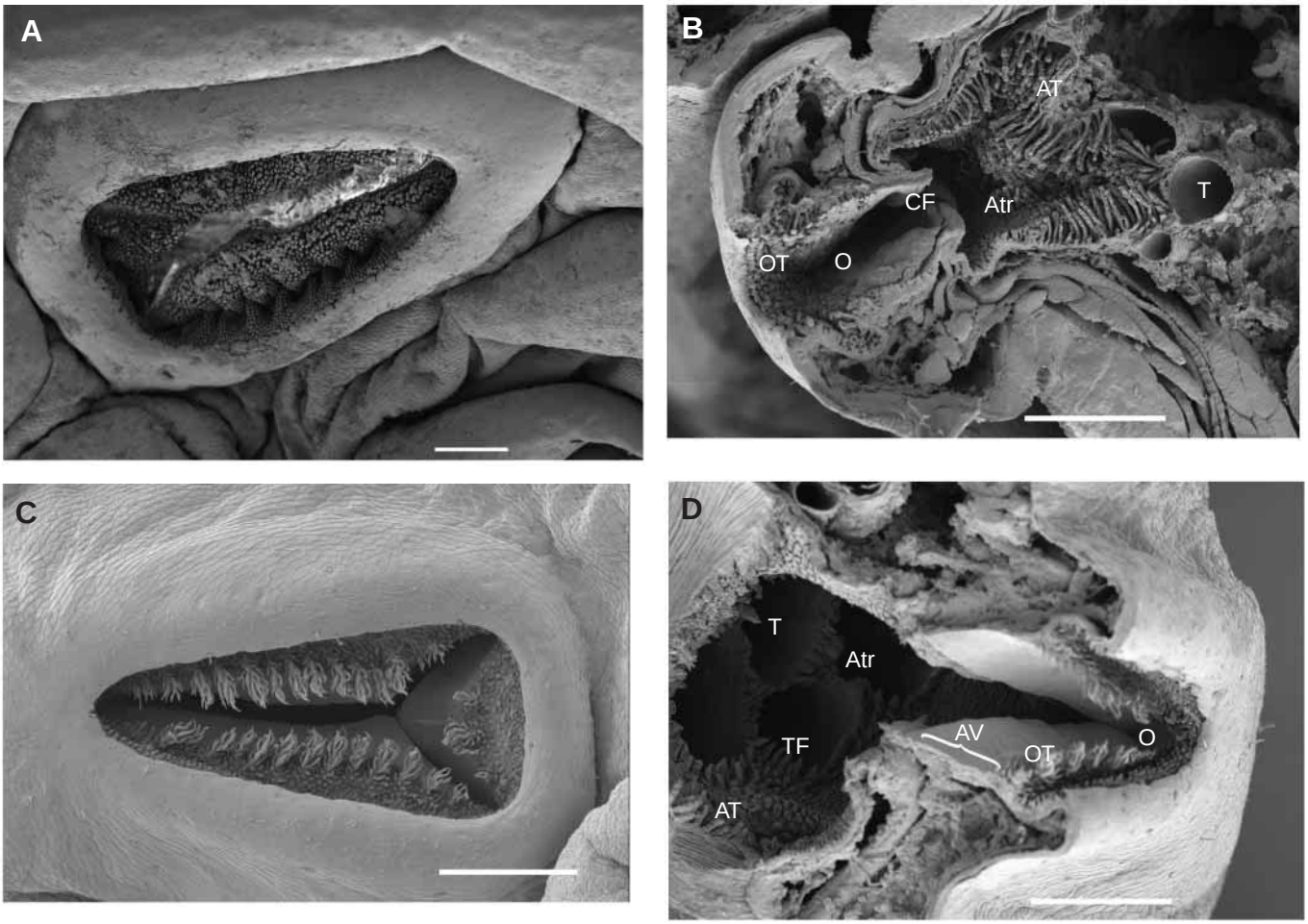

Fig. 3. (A) External view of a spiracle of *Cormocephalus elegans*. Note the uniform appearance of the ostial trichomes. (B) A tranverse section of the spiracle of *C. elegans*. Across the ostium (O), the ostial trichomes (OT) are separated from the atrial trichomes (AT) by a denuded cuticular fold (CF). The long atrial trichomes obscure the openings of the tracheae (T) in the atrium (Atr). (C) This external view of the spiracle of *C. morsitans* shows the elongated tufts of the ostial trichome. The three septa of the Y-shaped atrial valve are visible through the ostium. (D) A tranverse section of the spiracle of *C. morsitans*. Across the ostium (O), the ostial trichomes (OT) are separated from the atrial trichomes (AT) by the smooth denuded strip of cuticle of the atrial valve (AV). The shorter atrial trichomes leave the openings of tracheae (T) in the atrium (Atr) unobscured and only form a tracheal fimbrium (TF) around each opening. Scale bars, 100 μm.

trichomes form 12 tufts on both the dorsal and ventral edges of the triangular opening and five tufts on the posterior side (Fig. 3C). Directly behind these tufts there are broad (60 μm) strips of smooth cuticle separating the ostium from the tracheal atrium and forming a distinct Y-shaped opening. The inner surfaces of the tracheal atrium are lined with atrial trichomes much shorter than those observed in *C. elegans* (5–20 μm). These atrial trichomes do not cover the openings of the trachea. Each tracheal opening has a fringe (fimbrium) of atrial trichomes around the edge, and the various tracheal openings are clearly visible from the inside of the tracheal atrium (Fig. 3D).

Discussion

Centipede spiracles, gas exchange, mass scaling and metabolic rate

This study provides the first conclusive demonstration that certain species of scolopendromorph centipedes are indeed capable of closing their spiracles. Although several previous authors maintained that the structure of the spiracles was indicative of this ability (for reviews, see Lewis, 1981; Lewis et al., 1996), others have contested this idea (e.g. Curry, 1974), and it has certainly not made its way into the modern review literature (e.g. Little, 1990; Withers, 1992; Ruppert and Barnes, 1994).

The morphological observations, in conjunction with the normoxic and NAN respirometry, clearly indicate how complete spiracular closure is achieved (at least in *C. morsitans* and probably in *C. brevicornis*) and why this is unlikely in the other species. *Cormocephalus morsitans* possesses a valve system that allows tight closure of the spiracle, isolating the tracheal spaces from the external atmosphere. Strips of smooth, denuded cuticle separate the sub-ostial trichome layer from the atrial trichome layer, and it is these glabrous cuticular strips that form the Y-shaped atrial valve that ensures a secure seal during ostial contraction (Fig. 3C,D). In contrast, *C. elegans* has only vestiges of such cuticular strips, forming an uneven, cuticular fold between the sub-ostial and atrial trichomes (Fig. 3A,B). In this case, constriction of the ostium is unlikely to result in a tight seal and CO_2 leakage consequently occurs, as was evident in the interburst phase during respirometry.

Of the centipedes with occludible spiracles discussed by Lewis (1981) and Lewis et al. (1996), only some species from the genera *Scolopendra* and *Cormocephalus* had Y-shaped valves that appear to be homologous to those found in *C. morsitans* and *C. elegans*. In most of the other scolopendromorph taxa (e.g. *Cryptops hortensis*), the spiracular trichomes form a continuous layer from the ostium to the internal surface of the tracheal atrium (see Curry, 1974; Lewis, 1981; Lewis et al., 1996). If ostial contraction in a species such as *Cryptops hortensis* is possible, the continuous layer of trichomes is likely to prevent a secure seal, resulting in gas leakage. It therefore seems probable that some, but not all, species in the Scolopendromorpha are capable of closing their spiracles completely.

The morphological observations and the gas-exchange traces of the other centipede species examined here confirm previous ideas regarding the tracheal system and gas exchange in this group. Thus, *Scutigerina weberi* showed random diffusive CO_2 exchange patterns (Fig. 1E) consistent with the hypothesised operation of tracheal lungs and non-occludible spiracles (Lewis, 1981; Lawrence, 1983). Similarly, the Lithobiomorpha apparently have no spiracular closing mechanism (Lewis, 1981), and this certainly appeared to be the case in *L. melanops*, which showed considerable CO_2 emission rates during the ‘interburst’ phase (Fig. 1D).

Given the presence of occludible spiracles in at least one, but probably two, of the *Cormocephalus* species, it is perhaps not surprising that they showed evidence of a discontinuous gas-exchange cycle typical of some insects, soliphuges and ticks (see Lighton et al., 1993; Lighton, 1994, 1996; Lighton and Fielden, 1995, 1996; Harrison, 1997). Like DGCs in insects, those of the two *Cormocephalus* species are characterized, *inter alia*, by complete spiracular closure during the C-phase and an F-phase that predominates in terms of relative phase duration [compare Fig. 1A,B with Lighton (1992), Lighton and Fielden (1996), Davis et al. (1999) and Chown (2001)].

Scaling of the DGC phase characteristics in the centipedes was also generally positive and significant, as is the case in insects and soliphuges (see Lighton, 1991, 1996; Lighton and Fielden, 1996; Davis et al., 1999). However, a careful comparison of the exponents of the relationships between the groups is perhaps somewhat premature given that only two centipede species were examined here. Nonetheless, it is noteworthy that, unlike insects, the frequency of the centipede DGC scaled negatively with body mass (Table 3). Lighton (1991) showed that, as a consequence of similar scaling exponents for $\dot{V}_{CO_2}$ and for CO_2 emission volume, DGC frequency in insects does not vary with body mass, and Davis et al. (1999) substantiated this finding in a different group of insects. In the centipedes investigated here, O-phase CO_2 emission volume scales as $M^{1.103}$ and O-phase $\dot{V}_{CO_2}$ as $M^{0.926}$, resulting in DGC frequency scaling as $M^{-0.377}$.

These mass scaling considerations also have important implications for the scaling of $\dot{V}_{CO_2}$ and the metabolic rates of centipedes in general. To date, only two other studies of centipede metabolic rates have been undertaken: by Crawford et al. (1975) of *Scolopendra polymorpha* [546.07 μW (RQ=0.6) or 581.56 μW (RQ=0.84) and 1.5 g] and Riddle (1975) of *Nadabius coloradensis* [9.01 μW (RQ=0.6) or 9.59 μW (RQ=0.84) and 0.013 g]. When these values are combined with the data gathered here (Table 5) (and assuming an RQ of 0.84), the scaling relationship for centipede metabolic rate is $SMR=307M^{0.734}$ (SMR in μW and M in g). Lighton and Fielden (1995) used several hexapod and aranaeid taxa, whose metabolic rates scale identically as a conservative function of body mass (Schmidt-Nielsen, 1984; West et al., 1997), to generate a consensus scaling equation for arthropods, $SMR=906M^{0.825}$. These taxa have subsequently been designated ‘typical arthropods’ (Lighton et al., 2001). Ticks (Lighton and Fielden, 1995) and scorpions

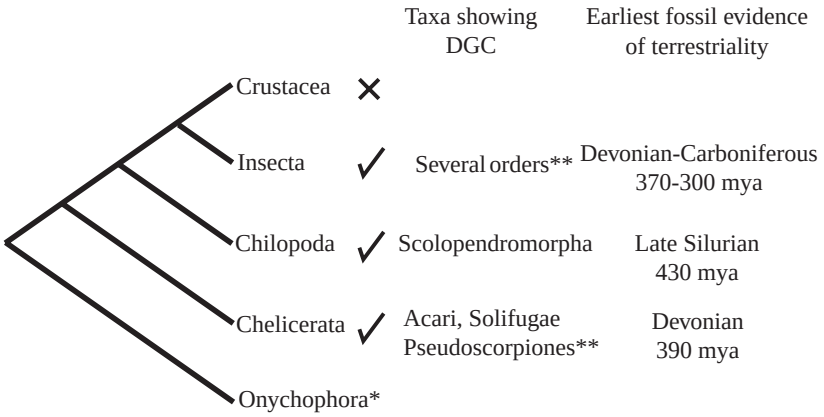

Fig. 4. An abbreviated cladogram (redrawn from Regier and Schultz, 1998; Strausfeld, 1998) showing the phylogenetic relationships between the major tracheated arthropod classes, the occurrence of discontinuous gas exchange cycles (DGCs) and the earliest known fossil evidence of terrestriality. *The tracheated Onychophora is shown as an outgroup. **See Lighton (1998). mya, million years ago.

(Lighton et al., 2001) are reported to deviate from the 'typical arthropods' in having 'anomalously' low metabolic rates, scaling respectively as SMR=132$M^{0.856}$ and SMR=237$M^{0.856}$. The present study adds centipedes as a third 'anomalously' low group. Scaling as SMR=307$M^{0.734}$, the slope (=scaling exponent) of the centipedes' relationship does not differ significantly (P<0.4) (see Sokal and Rohlf, 1995) from the slope of the scaling equation for 'typical arthropods', but the intercept (=scaling coefficient) is significantly (P<0.01) lower. Similarly, the mass-scaling exponents of ticks and scorpions do not differ from that found for the 'typical arthropods' (P<0.5 for both), but the metabolic rates of the former are significantly depressed (P<0.05). Thus, the metabolic rates of ticks, scorpions and centipedes are, respectively, approximately 15, 26 and 33 % of the metabolic rates of the so-called 'typical arthropods' (although, on the basis of the current data, there are no significant differences in metabolic rates of the 'anomalous' taxa, P=0.1). The physiological and ecological implications (*sensu* Lighton et al., 2001) of these differences in metabolic rate between the major arthropod taxa certainly warrant further investigation, but this is beyond the scope of the present study.

The evolution of the DGC

Recent investigations into the phylogenetic relationships of major arthropod taxa using various independent characters [molecular: Averof and Akam (1995a), Boore et al. (1995, 1998), Friedrich and Tautz (1995), Regier and Schultz (1997, 1998); developmental biology: Averof and Akam (1995b); nervous system anatomy: Osorio et al. (1995); Strausfeld (1998)] have all concluded that insects and crustaceans form the most derived sister group, preceded by the chilopods and chelicerates (Fig. 4). When the occurrence of the DGC is plotted on this 'consensus' phylogeny, the most parsimonious interpretation appears to be one of a single origin of the DGC in an ancestral arthropod, and the loss of the DGC in the Crustacea. However, tracheal breathing is a feature exclusive to the terrestrial arthropods (Pritchard et al., 1993). In addition, and with the exception of the insects, the tracheated taxa, and certainly their common ancestor, had marine origins and only later made the transition to a terrestrial lifestyle (Bergstrom, 1979; Kukalova-Peck, 1991; Pritchard et al., 1993; Labandeira, 1999). Thus, the likelihood of a single evolutionary transition to a DGC modality in a tracheal system seems low. Rather, it is likely that tracheal air-breathing and the associated morphological structures have evolved independently at least five times (Onychophora, Chelicerata, Myriapoda, Insecta and Isopoda) or possibly more frequently (Pritchard et al., 1993).

Periodic ventilation is also known in a wide variety of invertebrates and vertebrates (Harrison, 1997; Hustert, 1975; Innes et al., 1986; Innes and Taylor, 1986; Janiszewski and Otto, 1989; Komatsu, 1982; Ramirez and Pearson, 1989; Wilkens et al., 1989; McLean et al., 1995; Miyazaki et al., 1998; Bustami and Hustert 2000; Milsom, 2000; Smatresk et al., 2000; Szewczak 2000; Wilson et al., 2000). Therefore, it also seems likely that modification of the periodic component of the central pattern generator, to produce the quintessential DGC pattern characteristic of tracheated arthropods, has also occurred independently several times. In other words, there has been convergent evolution of discontinuous gas-exchange cycles in the Arthropoda.

In conclusion, we have shown that at least some centipede species in the Scolopendromorpha can close their spiracles, that these species have DGCs similar to those found in insects, soliphuges and ticks and that the DGC has evolved independently at least four times in the Arthropodae (Acari and Pseudoscorpiones being counted as two). This suggests that the DGC may well have an adaptive function. To determine the possible advantages or environmental correlates of this gas-exchange modality will require substantial species-level comparative and experimental work.

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