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The origins of tetrapulmonate book lungs and their significance for chelicerate phylogeny

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Summary

Tetrapulmonate arachnids have two pairs of lamellate respiratory organs on opisthosomal segments 2 and 3. Larval xiphosurans have a full complement of opisthosomal segments, but a reduced number of opisthosomal appendages. A hypothesis is proposed in which the tetrapulmonate condition arose in an arachnid ancestor through the progenetic retention of the reduced number of larval gills. Significantly, tetrapulmonate arachnids cannot easily be derived directly from xiphosurans, which lack book gills on opisthosomal segment 2 and, likewise, scorpions and eurypterids also lack respiratory organs on these segments. As such, tetrapulmonates are the only chelicerates to retain the plesiomorphic state of a lamellate respiratory organ on the genital segment (opisthosomal segment 2). Either tetrapulmonates are the sister group to all other chelicerates, or this loss of a book gill/lung pair must be convergent between scorpions, eurypterids and xiphosurans.

Introduction

The arachnid taxon Tetrapulmonata Shultz, 1990, includes those orders which have, at least primitively, two pairs of book lungs, i.e. Trigonotarbida, Araneae, Amblypygi and Uropygi (Selden *et al.*, 1991). It includes the most familiar living arachnids (spiders) and one of the most abundant Palaeozoic groups (trigonotarbids). Among the arachnids, book lungs are widely accepted as the most primitive mode of terrestrial respiration (Levi & Kirber, 1976), as opposed to tracheal systems. Book lungs have long been demonstrated to be homologues of the book gills of xiphosurans (horse-shoe crabs), a proposal first credited to Van Beneden (1871). Though there is no evidence to derive arachnids directly from any particular xiphosuran, this paper proposes a model in which the tetrapulmonate condition could have arisen from a xiphosuran-like ancestor by progenesis. This paper also demonstrates that only tetrapulmonates retain a pair of lamellate respiratory organs on the genital segment and that

this is a plesiomorphic state not seen in other Chelicerata. The implications of this are significant, suggesting that tetrapulmonates could be the sister group of the remaining chelicerates.

Tetrapulmonate respiratory organs

The gross anatomy and fine structure of the tetrapulmonate book lung has been described by numerous authors (e.g. Foelix, 1982; Hexter, 1982; Reisinger *et al.*, 1990). Essentially each lung comprises an invagination of the cuticle from a narrow spiracle into an atrium, from which a number of thin-walled lamellae arise. Embryological evidence convincingly demonstrates that the arachnid book lung is homologous with the xiphosuran book gill (see Anderson (1973) for a review), since both structures are derived from projecting lamellae which develop from the posterior wall of opisthosomal limb buds. More recently, it has been demonstrated on anatomical evidence that the book lungs in Uropygi are also borne on modified

appendages termed lung opercula (Shultz, 1993) (Fig. 1).

These arachnid lung opercula are homologous with the gill opercula in xiphosurans and the Blatfüsse (gill opercula) in eurypterids (sea scorpions); eurypterids probably had xiphosuran-like book gills attached to these Blatfüsse (Selden, 1985; Manning & Dunlop, 1995). The ventral sclerites, traditionally interpreted as opisthosomal sternites 2 and 3, in uropygids are highly modified, lung-bearing appendages (this is probably also true for amblypygids), while the true sternites 2 and 3 are highly reduced elements concealed beneath these opercula (Shultz, 1993) (Fig. 1). By this reasoning, it seems likely that "sternites" 2 and 3 in the extinct Trigonotarbida are also opercula (a convention adopted by Dunlop (1995, 1996)). Morin (1887) first noted that spider lung opercula were formed from appendage rudiments during embryology and these sclerotized lung opercula are almost certainly highly derived appendicular elements in spiders as well.

Development of xiphosuran book gills

Embryological studies have proved invaluable in determining the homology of chelicerate

respiratory organs. Kingsley (1885, 1892), one of the first to study the embryology of *Limulus*, noted that, in addition to the chilaria, two pairs of opisthosomal appendages develop first: the operculum and the first gill-bearing or branchial appendage. He figured this developmental stage (Kingsley, 1885, pl. 37, figs. 12, 14) which represents the so called "trilobite larva" of the horseshoe crabs. Iwanoff (1933) described a slightly different pattern for the SE Asian xiphosuran *Carcinoscorpius* (in his paper still called "*Limulus*"). He described and figured the initial development of the operculum and the next two pairs of gill-bearing appendages (Iwanoff, 1933, fig. 55), this pattern of development being cited in reviews of xiphosuran embryology such as Fage (1949) and Anderson (1973). Itow (1986, fig. 3a) also showed that the first instar larvae of the third extant xiphosuran, *Tachypleus*, have only four pairs of opisthosomal appendages: (1) the chilaria, (2) the operculum, (3) a pair of full-sized first branchial appendages, and (4) a pair of much smaller second branchial appendages (Fig. 2). In xiphosurans, additional branchial appendages are added with subsequent moults to give the adult complement of a genital operculum plus five branchial appendages. It is possible that Kingsley (1885, 1892) overlooked the second, smaller, branchial appendages in his

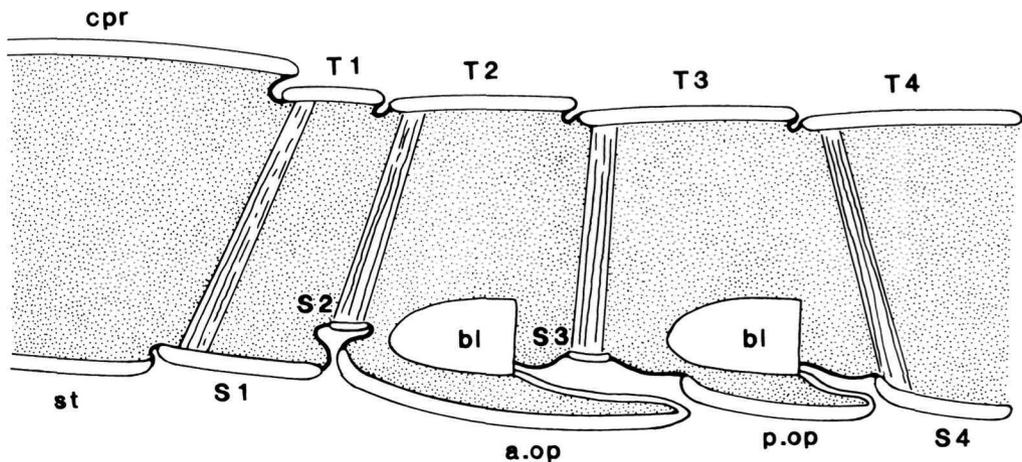


Fig. 1: Diagrammatic longitudinal section through a tetrapulmonate arachnid, based on the uropygid studies of Shultz (1993). Tergites can be matched to sternites by their dorso-ventral musculature which shows that sternites 2 and 3 are highly reduced and that the book lungs are in fact borne on modified appendages, the opercula, homologous with the gill opercula of xiphosurans. a.op = anterior operculum, bl = book lung, cpr = carapace, p.op = posterior operculum, S = sternite with number, st = sternum, T = tergite with number.

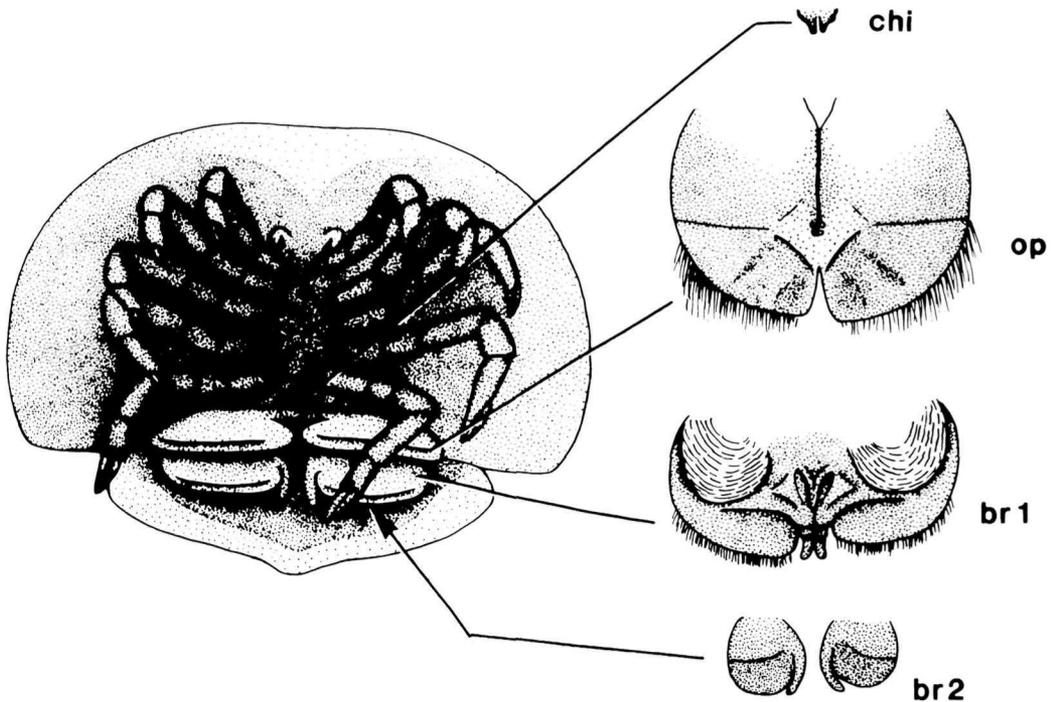


Fig. 2: Ventral view of the larva of the xiphosuran *Tachypleus* sp. (after Itow, 1986, fig. 2). Opisthosoma slightly flexed. Detail of the four opisthosomal appendages (after Itow, 1986, fig. 3) also shown. chi = chilaria, op = genital operculum, br1 = first branchial (i.e. gill-bearing) appendages, br2 = second branchial appendages. Gills only evident on first branchial appendages at this developmental stage.

studies of *Limulus*, as they are concealed beneath the larger pair of first branchial appendages.

Origins of the tetrapulmonate condition

Xiphosurans, scorpions and the extinct eurypterids have all been proposed at one time or another as outgroups for the other arachnids, and all have four or five pairs of opisthosomal respiratory organs. However, Tetrapulmonata have only two pairs of respiratory organs. If we are to understand how the tetrapulmonate condition arose we could look for a heterochronic change; the importance of heterochrony in arthropod evolution was reviewed by Minelli & Fusco (1995).

Evidence from the development of extant xiphosurans indicates that in the early stages of their life cycle they lack the full complement of opisthosomal appendages. Most significantly, it is the gonopore-bearing operculum

(opisthosomal segment 2) and the first branchial appendage (opisthosomal segment 3) which are most completely developed in larval xiphosurans (Kingsley, 1885; Iwanoff, 1933; Itow, 1986). These appendages correspond to the lung-bearing appendages of tetrapulmonate arachnids; their appendicular nature in arachnids was demonstrated by Shultz (1993) (Fig. 1). All tetrapulmonates lack appendages on opisthosomal segment 1 (where the chilaria are very small in larval xiphosurans), and Shultz (1990) argued that this appendage loss was synapomorphic for all arachnids. Some tetrapulmonates (trigonotarbid, uropygid and amblypygid) also lack appendages on opisthosomal segment 4 and succeeding segments; larval xiphosurans have an underdeveloped opisthosomal appendage 4 which lacks gill lamellae.

An obvious exception to this are the spinnerets of spiders, which were regarded by Shultz (1987), among others, as appendages of opisthosomal segments 4 and 5, possibly representing the modified gonopods of these segments.

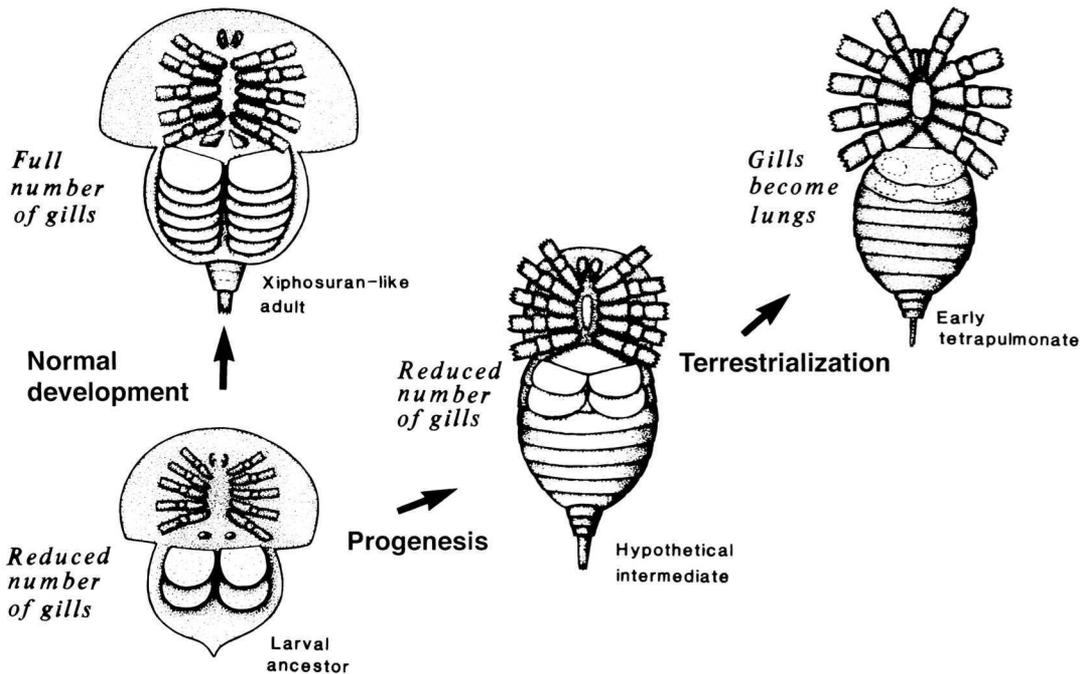


Fig. 3: Hypothetical model by which the tetrapulmonate condition arises through progenesis of a xiphosuran-like ancestor, such that only the gill-bearing appendages of segments 2 and 3 remain. These gill opercula subsequently suture onto the opisthosoma to form the anterior and posterior opercula (see Fig. 1) as the gills evolve into lungs during terrestrialization. This model assumes a gill on opisthosomal segment 2 of the hypothetical ancestor.

Marples (1967) proposed that the median spinnerets of spiders were glandular in origin, not appendicular, and homologous with the epiandrous glands on the genital segment of male spiders (see Shultz, 1987, for evidence against this). Even so, Marples (1967) still regarded the lateral spinnerets of spiders as appendicular and noted the problem that if spinnerets are primitively retained opisthosomal appendages on segments 4 and 5, why are they absent on these segments in all other tetrapulmonates? Marples (1967) discussed two theories: (1) spiders are more primitive than other tetrapulmonates, which later lost these appendages; or (2) these appendages are suppressed in all tetrapulmonates, but present in the embryo where spiders progenetically retain them and develop them as spinnerets, i.e. paedomorphic appendages. Marples (1967) did not like either of these suggestions. There is clearly further work to be done on the origins of

spinnerets, but this paper will concentrate on the appendages bearing respiratory organs.

Progenesis?

A hypothesis is presented (Fig. 3) in which the tetrapulmonate condition of two pairs of respiratory organs arose by progenesis from xiphosuran-like ancestors. In this hypothesis, living larval xiphosurans, with their reduced opisthosomal appendages, suggest how an aquatic early chelicerate/arachnid could have retained its larval condition and failed to develop full opercula on opisthosomal segment 4 and the segments posterior to this. These animals had only two pairs of gills as adults (there are no gills on the xiphosuran genital operculum, but see below for a discussion of gills on segment 2) and with terrestrialization these branchial appendages sutured onto the ventral body wall

and the gills became the two pairs of book lungs in the Tetrapulmonata (Fig. 3).

However, structures can be lost by mechanisms other than progenesis. One criticism of the hypothesis could be that arachnids actually have more opisthosomal segments (12) than extant xiphosurans (9), though fossil xiphosurans had up to 11 opisthosomal segments (Anderson & Selden, 1997). A progenetic loss of appendage-bearing segments in an early tetrapulmonate might be expected to produce an animal with fewer opisthosomal segments than its ancestor. However, Minelli & Fusco (1995) noted that segmentation of structures such as the central nervous system often precede the appearance of appendages. In evidence of this, they cited Itow's (1986) observation that larval xiphosurans have the full, adult number of ganglia in their central nervous system and his interpretation that the full number of body segments is present in these larvae, although only four pairs of opisthosomal appendages develop initially. What this means for tetrapulmonate evolution is that a paedomorphic animal could exist with a reduced number of opisthosomal appendages, but a full complement of opisthosomal segments. It is also worth noting Jeram's (1994) observation that juvenile specimens of the Carboniferous scorpion *Pulmonoscorpius* had only three abdominal plates (i.e. opercula) but a full complement of tergites, and his suggestion that an additional abdominal plate was added later during development to give the adult number of four. This developmental pattern of adding opisthosomal appendages may be widespread among chelicerates, while Firstman (1973), based on comparative studies of the endosternite, also argued that arachnid evolution has involved neoteny and adaptive radiation from neotenic ancestors.

The Siluro-Devonian fossil record of non-scorpion arachnids mostly comprises small taxa (Shear & Kukulová-Peck, 1990), typically 5–10 mm long, whilst most fossil scorpions of this age are an order of magnitude larger (Kjellesvig-Waering, 1986). The earliest xiphosurans were in the 5–10 cm size range (Anderson & Selden, 1997), whereas many eurypterids were considerably larger. Although the arachnid fossil record is incomplete, if the early tetrapulmonates evolved through progenesis from a "merostome-like" ancestor it would not be surprising if they

were relatively small, inconspicuous animals. Current phylogenetic models (Dunlop & Selden, 1997) predict arachnid origins in the Ordovician. Progenesis might explain why we have no pre-terrestrial or pre-Silurian fossil record for the oldest arachnids; their small size conferred on them a poor preservation potential.

Phylogenetic implications

As noted above, xiphosurans do not have gills on their operculum (opisthosomal segment 2, the genital segment), but tetrapulmonates have book lungs in this position. A paedomorphic xiphosuran, in the model above, would evolve into something with only a single pair of gills/lungs on opisthosomal segment 3. So does this disprove the model? Well, within the chelicerates, only the tetrapulmonates retain a lamellate respiratory organ on their genital segment. In xiphosurans this segment bears the genital operculum only. In scorpions this segment bears a small pair of genital opercula. In eurypterids there is a genital operculum composed of 2 fused appendage pairs where the presence of lamellate gills and the segment they belong to is equivocal (Fig. 4), though they do have a branchial chamber with a Kiemenplatte associated with this fused genital operculum (Manning & Dunlop, 1995). What this implies is that it is difficult to derive tetrapulmonates from a xiphosuran, scorpion or eurypterid clade without invoking a reversal to re-acquire the gills or lungs on segment 2. It also means that the book lungs in scorpions are not serial homologues of the book lungs in tetrapulmonates, because they belong to different opisthosomal segments (Fig. 4). Outgroup comparison of chelicerates with well-preserved fossil arachnomorphs (trilobites, etc.) suggests that the plesiomorphic state should be paired respiratory organs on each opisthosomal segment (e.g. Størmer, 1944), including the genital segment.

If loss of gills on opisthosomal segment 2 were synapomorphic for Xiphosura + Eurypterida + Scorpionida, then Tetrapulmonata, in retaining this character, would emerge as a sister group to these three taxa (Fig. 5a, but see Shultz, 1990, for a list of synapomorphies for a monophyletic Arachnida). In Figure 5a, scorpions and eurypterids are interpreted as sister groups on the synapomorphy of a five-segmented

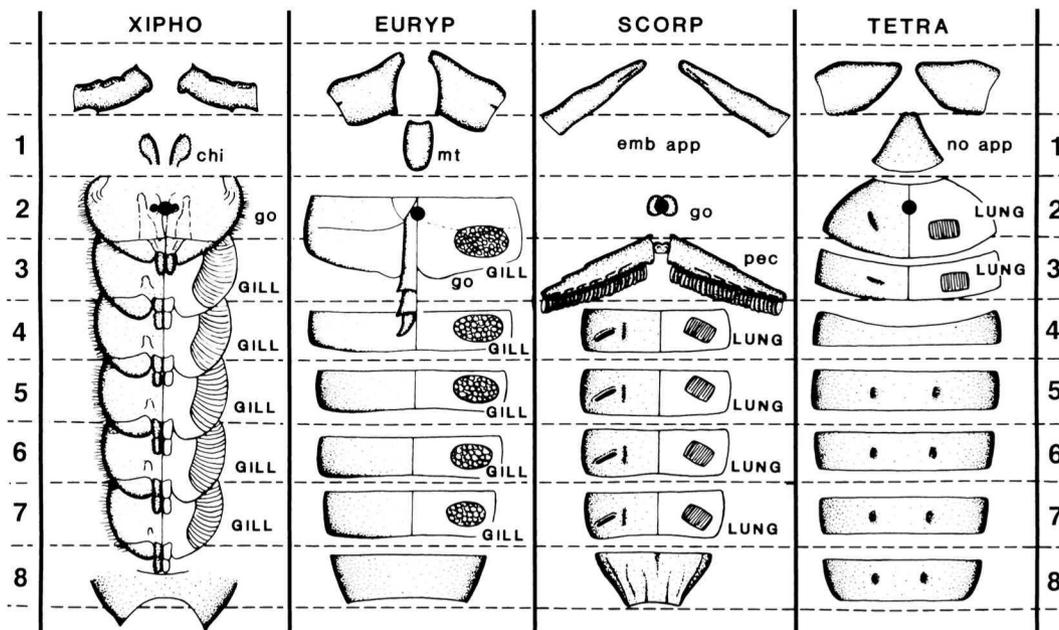


Fig. 4: Suggested homology of the anterior ventral opisthosomas in xiphosurans (XIPHO), eurypterids (EURYP), scorpions (SCORP) and tetrapulmonates (TETRA), the latter represented by a uropygid. Not to scale. Note that only tetrapulmonates retain a lamellate appendage on the second opisthosomal (genital) segment. Gill chambers in eurypterids identified by presence of Kiemenplatten (gill tracts) and first gill on the fused genital operculum assumed to belong to segment 3. Opisthosomal segments numbered and position of gonopore indicated by black circle. chi = chilaria, emb app = appendages resorbed in embryo, go = genital operculum, mt = metastoma, no app = no appendages, pec = pectines.

postabdomen. Shultz (1990) rejected a relationship between scorpions and eurypterids, claiming such proposals were only based on vague morphological similarities. A synapomorphy for these groups is here explicitly proposed. Alternatively, if tetrapulmonates represent more derived chelicerates than in Figure 5a (e.g. a more traditional figure, 5b, based on Weygoldt & Paulus (1979)), then the loss of the gill from opisthosomal segment 2 in xiphosurans, eurypterids and scorpions must be a convergent phenomenon.

The model presented in Figure 5a is undoubtedly controversial (essentially arguing that spiders and their relatives are sister group to supposedly primitive chelicerates like horseshoe crabs!) and can be justifiably criticized for ignoring the tracheate arachnids and being argued on a single character, albeit an important one. Figure 5a is similar to the model of Bergström (1979) in which arachnids (excluding

scorpions) could be traced to a very ancient origin forming a separate lineage from the "merostomes". Figure 5a should not be regarded as a final phylogeny, but neither of the most recent cladistic analyses of chelicerates (Weygoldt & Paulus, 1979; Shultz, 1990) noted the need to reverse the loss of a book gill to obtain the tetrapulmonate condition in their phylogenies.

The book lung itself is evidently a convergent terrestrial adaptation in tetrapulmonates and scorpions (Purcell, 1910; Shultz, 1990). What the model does not address is the position of the tracheate arachnid orders where a number of different body plans are found: e.g. spiracles on opisthosomal segment 2 in opilions, segments 3 and 4 in pseudoscorpions, segments 3–5 and the prosoma in solifuges, spiracles adjacent to leg coxae in ricinuleids, dorsal spiracles in opilions (mostly after Shultz, 1990). Tracheal systems are evidently derived among

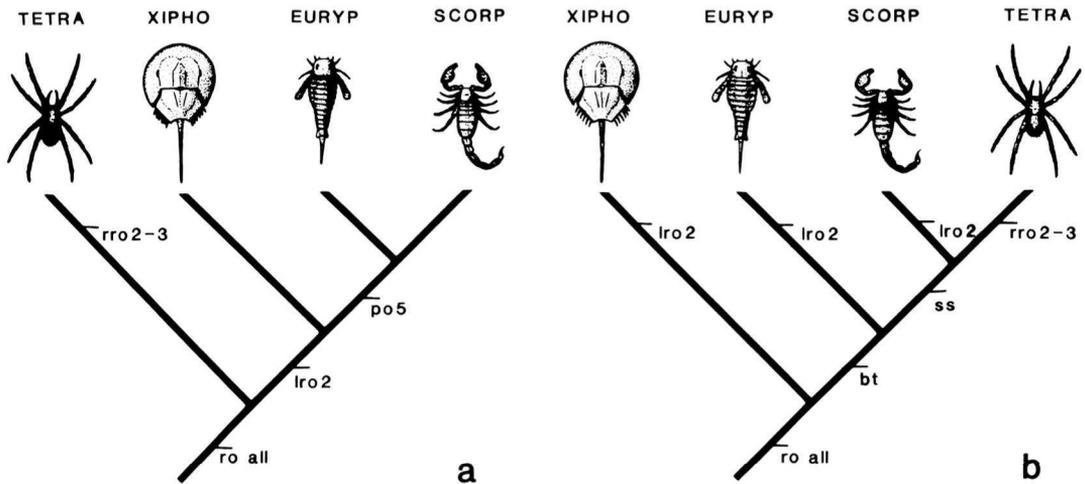


Fig. 5: **a** Hypothetical chelicerate phylogeny based on the distribution of respiratory organs. In this model only tetrapulmonates (TETRA) retain the plesiomorphic condition of respiratory organs on segment two, and their loss in the other taxa, Xiphosura (XIPHO), Eurypterida (EURYP) and Scorpionida (SCORP), is derived and synapomorphic. **b** A more traditional chelicerate phylogeny after Weygoldt & Paulus (1979) and Shultz (1990) showing convergent loss of respiratory organs on opisthosomal segment 2 in xiphosurans, eurypterids and scorpions. ro all = respiratory organs on all opisthosomal segments (plesiomorphic state), rro 2-3 = restriction of respiratory organs to opisthosomal segments 2-3, lro 2 = loss of respiratory organs on opisthosomal segment 2. Other selected synapomorphies: bt = basitarsus, po 5 = postabdomen of 5 segments, ss = slit sensilla.

arachnids and not all of them can be convincingly homologized with book lungs and book gills. The hypotheses in Figure 5 are presented for further discussion.

Conclusions

It remains possible that the tetrapulmonate condition arose relatively late in arachnid evolution, e.g. a common ancestor to a monophyletic arachnid clade with multiple pairs of gills/lungs (but before the Lower Devonian when we have the first evidence for two pairs of book lungs (Claridge & Lyon, 1961)). That said, I have tried to present a coherent argument that tetrapulmonates cannot easily be derived from xiphosurans, eurypterids or scorpions, all of which have the more derived state of no respiratory organs on opisthosomal segment 2. It is more parsimonious to assume a more distant chelicerate ancestor which possessed respiratory organs on all opisthosomal appendages. This implies that tetrapulmonates are sister group to the other chelicerates. Studies of xiphosuran ontogeny provide a mechanism in the most

“primitive” living chelicerates by which gill-bearing appendages could become restricted in this ancestor to the anterior opisthosomal segments by progenesis.

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